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THE LARVA OF *POLYTHORE SPAETERI* BURMEISTER & BÖRZSÖNY, WITH COMPARISON TO OTHER POLYTHORID LARVAE AND MOLECULAR SPECIES ASSIGNMENT (ZYGOPTERA: POLYTHORIDAE)

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The larva from the area of Panguana (Huanuco prov., Peru) is described. This constitutes the first description of a Polythore. P-distance measuring of a 790 bp long fragment of the mitochondrial COI gene was used as a tool for the assignment of the larva. The low degree of sequence divergences between larval and imaginal COI sequences leaves no doubt about conspecificity. The use of scanning electron microscopy gives an impression of some morphological characters not mentioned so far concerning polythorid larvae. Comparison of the *P. spaeteri* larva with the few currently available descriptions of polythorid larvae shows that characterisation of the larvae at generic level is not possible until more larval specimens of the family are examined.

INTRODUCTION

The genus *Polythore* Calvert, 1917 is mainly distributed in western South America, where it is most common in Peru (BICK & BICK, 1986). BURMEISTER & BÖRZSÖNY (2003) described a new Peruvian species, *P. spaeteri*, which is currently the only record of Polythoridae from the region of Panguana (an Amazonian area close to the Andes, elevation 250 m, covered with primary rain forest). This species is well distinguishable from others in the genus by wing colour pattern and by characters of the male genitalia.

During a systematic search for the immature stages of *P. spaeteri*, larvae were found clinging to the lower surfaces of larger stones in a small forest stream with relatively strong current. The larvae were equipped with lateroventral, serial gill-like appendages on the abdomen, which is a special feature of the larvae of only

two families within the Odonata: the Polythoridae and the oriental family Euphaeidae (LIEFTINCK, 1962; WICHARD, 1979; BECHLY, 1998).

So far, larval descriptions of only six polythorid species and subspecies exist: *Cora chirripa* Calvert, 1907 (CALVERT, 1911), *C. cyane* Selys 1853 (DE MARMELS, 1982), *C. marina* Selys, 1868 (NOVELO-GUTIERREZ & GONZALES-SORIANO, 1985), *Chalcopteryx rutilans* Rambur, 1842 (DOS SANTOS & COSTA, 1987), *Chalcothore montgomeryi* Rácenis, 1968 (DE MARMELS, 1988) and *Euthore fastigiata meridana* Selys, 1879 (DE MARMELS, 1995).

Because no other Polythoridae were found in the study area, the likelihood of species identity with the imagines of *P. spaeteri* appeared to be high. Attempts at keeping collected larvae alive until their last moult were unsuccessful. Thus, another method, making use of molecular techniques in taxonomy, was applied to test for species identity. A fragment of the gene coding for the mitochondrial cytochrome oxidase subunit I (= COI) was compared between larvae and imagines. *Calopteryx splendens* (Harris) was used as reference species. More adequate reference species (possibly from the same family or even the same genus) could have advanced the comparability and the recognition of presumptive species boundaries, but at the time of this study *C. splendens* offered the only material readily available and showing good PCR results. The families Calopterygidae and Polythoridae are generally placed into the same super-family Calopterygoidea (e.g. FRASER, 1957; REHN, 2003).

ZLOTY et al. (1993) made first attempts to use protein electrophoresis to identify *Hetaerina* larvae. Therefore they are the founders in using molecular techniques in odonatology. We refined their approach to the level of matching DNA sequences between larvae and adults. As far as we know, this method of species identification is applied for the first time within Odonata.

The following description of the presumptive larva of *P. spaeteri* is the first of its kind in the genus *Polythore*. It contains drawings of the habit and scanning

Table I
Morphological voucher specimens and extracted DNA deposited in the frozen DNA and tissue collection of ZSM

Specimen	DNATAX-ID	Collecting data
P. spaeteri imago	DNATAX02710	
P. spaeteri imago	DNATAX02711	
P. spaeteri imago	DNATAX02713	Peru, Huanuco prov., nr Rio Yuyapichis (Llullapichis),
P. spaeteri larva	DNATAX02714	Panguana, 9°37′S 74°56′E; 06-IV - 17-IV-2003
P. spaeteri larva	DNATAX02716	
P. spaeteri larva	DNATAX02718	
Calopteryx splendens imago (reference)	DNATAX02712	Germany, Bavaria, Munich, Obermenzing, ZSM grounds, 2-VI-2003

electronic microscope (SEM) photographs of some special features. A diagnosis against the hitherto described larvae of other polythorid species is presented.

MATERIAL & METHODS

SPECIMENS. – 19 larvae were collected in Peru, Huanuco prov., near the Rio Yuyapichis (Llull-apichis), at the biological station Panguana, 9°37'S and 74°56'E, between 06-IV and 17-IV-2003, H., J. & E.-G. Burmeister leg. Nine larvae were preserved in approx. 100% ethanol for molecular analysis, ten in 70% ethanol. All are stored in the ZSM (= Zoologische Staatssammlung München). Nine larvae (in approx. 100 % ethanol) were used for the molecular identification, five of these (3♀, 2♂), preserved in good condition, were selected for the morphological description. Imagines were collected by E.-G. Burmeister at the same locality, between 28-IX and 06-X-2000, and by H., J. and E.-G. Burmeister between 06-IV and 17-IV-2003. The imagines of the reference-species, *Calopteryx splendens*, were collected by E.-G. Burmeister in the surroundings of the ZSM, on 02-VI-2003.

DESCRIPTION. – Some parts were dissected and examined in a LEO 1430VP scanning electron microscope (SEM) at 15 kV after critical-point drying in the CPD 030 apparatus (BAL-TEC), with CO_2 as exchange medium and subsequent coating with gold in a sputter-coater apparatus (Polaron, SEM COATING SYSTEMS) at the following parameters: 2.4 kV, 20mA and sputtering-time 120 sec., resulting in a coating thickness of approx. 25 nm.

MOLECULAR ANALYSES. – Total genomic DNA of three *P. spaeteri* imagines and nine larvae was extracted from thoracic muscles of whole individuals by means of the DNeasy Tissue Kit (QIAGEN) according to the manufacturer's protocols. The three larvae showing the highest effects on DNA extractions were used for the further procedure. They are deposited in the DNA-TAX DNA and voucher collection at ZSM, along with the imagines of *P. spaeteri* and *C. splendens* (collection numbers, Tab. I).

Table II

Positions of sequence divergences (in boldface) among and between *P. spaeteri* larvae and imagines, respectively. – [Positions of sequence divergences (in boldface) among and between *P. spaeteri* larvae and imagines, respectively]

DNATAX- ID		Positions of divergent nucleotides, relating to the complete COI-gene																	
	Base										1	1	1	1	1	1	1	1	1
	position	7	7	8	9	9	9	9	9	9	1	1	1	3	3	3	3	3	3
		9	9	0	8	8	8	9	9	9	6	6	6	6	6	6	8	8	8
		8	9	0	5	6	7	6	7	8	4	5	6	5	6	7	0	1	2
	Codon																		
	position	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3	1	2	3
DNATAX02710	*P.spaeteri*-																		
	imagines	G	G	T	C	T	A	A	G	**C**	G	G	A	A	T	**T**	C	T	**T**
DNATAX02711		G	G	T	C	T	A	A	G	T	G	G	**G**	A	T	**T**	C	T	C
DNATAX02713		G	G	T	C	T	A	A	G	T	G	G	A	A	T	**T**	C	T	C
DNATAX02714	*P.spaeteri*-																		
	larvae	G	G	T	C	T	A	A	G	T	G	G	A	A	T	**C**	C	T	C
DNATAX02716		G	G	**C**	**T**	T	A	A	G	T	G	G	A	A	T	**C**	C	T	C
DNATAX02718		G	G	T	C	T	A	A	G	T	G	G	A	A	T	**C**	C	T	C
coded amino acid		Gly			Leu			Ser			Gly			Ile			Leu		

For the amplification of the 790 bp long fragment of the COI gene, the following universal insect primers were selected from SIMON et al. (1994):

C1-J-2195 (forward):
5′ TTGATTTTTTGGTCATCCAGAAGT 3′
and TL2-N-3014 (reverse):
5′ TCCAATGCACTAATCTGCCATATTA 3′

The PCR was performed on a PTC 220 DYAD thermocycler (MJ RESEARCH) with a total reaction volume of 25 µl using the Expand-PCR System (ROCHE DIAGNOSTICS) at the following parameters: 25 pmol of each primer; 25 pmol DNTPs; 12.5 pmol $MgCl_2$ and 0.88 units of taq-polymerase. The PCR ran at the following parameters: 94°C for 4 min; 45 cycles at 94°C for 1.5 min; 48°C for 1min; 72°C for 1.5 min and a final elongation step at 72°C for 3 min.

The results of the PCR were visualised under UV light using ethidium-bromide stained agarose gels. The following purification of the PCR products was performed using the MinElute PCR-purification kit (QIAGEN), according to the manufacturer's protocols. The amplified dsDNA fragment of the COI gene of each specimen was used as template for the sequencing reaction with the respective PCR primers, using the Ready Reaction DyeDeoxy Terminator Cycle Sequencing Kit ("Big Dye", APPLIED BIOSYSTEMS) at the following parameters: 94°C for 2 min; 25 cycles at 94°C for 20 sec; 52°C for 10 sec; 70°C for 4 min. The fragments were sequenced in both directions. The filtered and resuspended products were electrophoresed on an ABI 377 automated sequencer. The sequences contained in the ABI files were checked manually; sequences of the *P. spaeteri* imagines were aligned to those of the larvae, and the sequences of both were aligned to that of the reference species, *C. splendens*. The pairwise distances between larval and adult sequences were calculated with the formula $p = n_d / n_t$ (= p-distance measuring, whereas n_d is the number of nucleotide divergences between two sequences, and n_t is the total number of compared nucleotides; see HEBERT et al., 2003b)

MOLECULAR ANALYSES

In the 790 bp long COI fragment compared between *P. spaeteri* imagines and larvae, sequence divergence was almost negligible. Variance among the larval and imaginal sequences, respectively, was small as well (see Tab. II).

Positions divergent between *P. spaeteri* and the reference species, *C. splendens*, are not given in detail here, but the p-distance of 22.4% shows the expected divergence (see Tab. III).

Table III
p-distance values according to the formula $p = n_d / n_t$ after HEBERT et al. (2003b); see "Material & methods"

p-distance among larvae	p-distance among imagines	p-distance between larvae and imagines	p-distance to reference
0.25 %	0.38 %	0.76 %	22.4 %

The sequences are deposited in EMBL/GenBank under accession numbers: AM_180640 for DNATAX02710, AM_180641 for DNATAX02711, AM_180642 for DNATAX02713, AM_180643 for DNATAX02714, AM_180644 for

DNATAX02716, AM_180645 for DNATAX02718, and AM_180646 for DNATAX02712.

On the basis of these results (see also Discussion) the larva described in this paper will be referred to as *P. spaeteri*-larva from now on.

DESCRIPTION OF THE *P. SPAETERI* LARVA

Figures 1-8

Measurements (in mm): Body length: ~15.0, presumptive ultimate instar (~11.0, presumptive penultimate instar); length of the paraprocts: 3.5 (2.8); length of the epiproct: 3.0 (2.3); length (with-

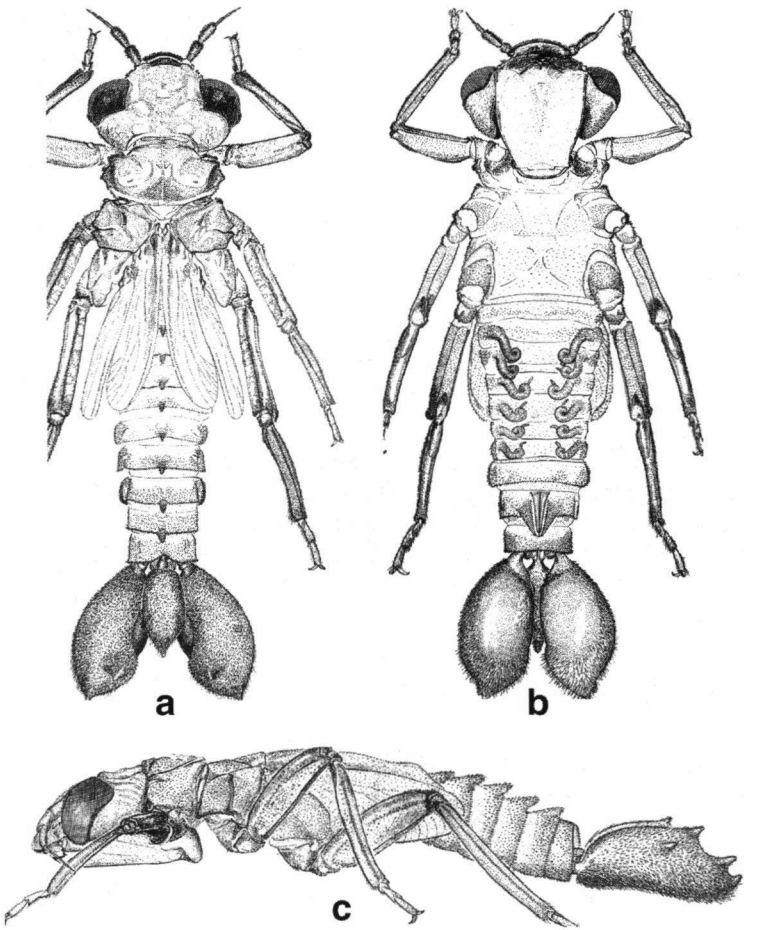
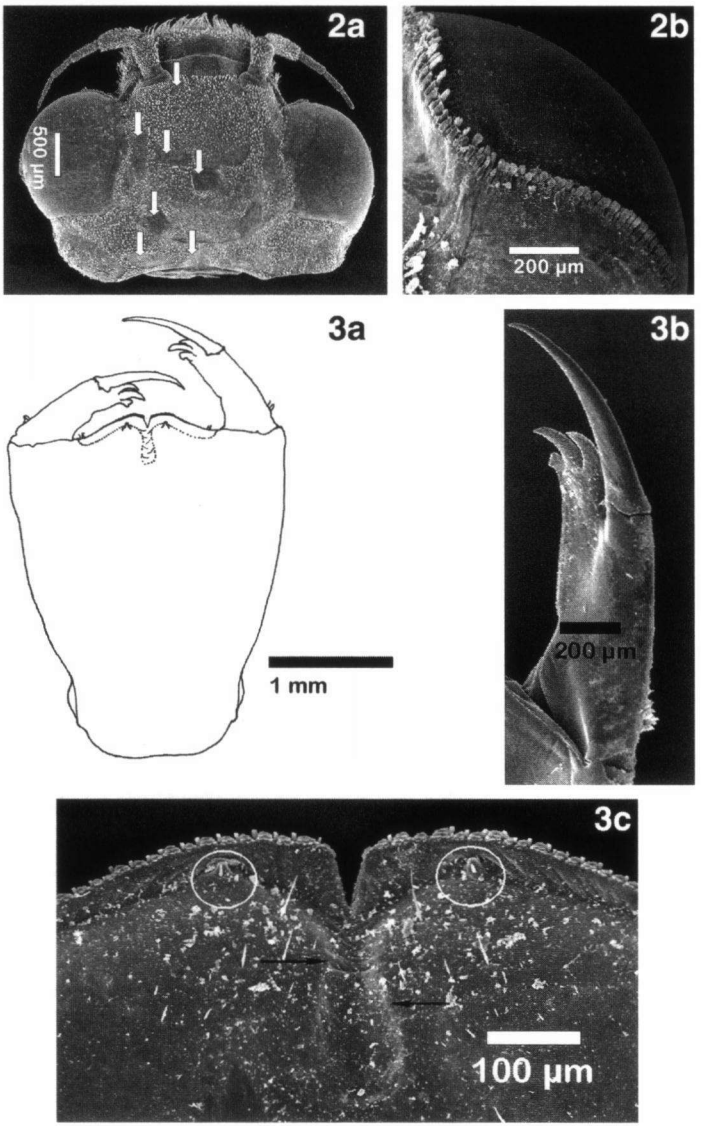


Fig. 1. *Polythore spaeteri*, habit of the presumptive penultimate instar in dorsal (a), ventral (b) and lateral (c) views. – (drawings by R. Kühbandner)



Figs 2-3. *Polythore spaeteri*, larval structural features: (2) (a) head, dorsal view (arrows indicate 7 of the 13 symmetrically arranged bare spots); (b) fringy incised integumental border close to compound eyes, ventral view; – (3) (a) labium, dorsal view (dotted lines indicate a slight embossment with a spike-like tooth on each side); (b) right labial palp, dorsal view, with a movable claw on the right; (c) margin of the labial prementum (white circles indicate spike-like teeth, presumptive relics of parts of the former second maxillae; arrows point to the corrugated presumptive fusion line of the former second maxillae; on both sides of the fusion line, small, rather symmetrically arranged setae are visible).

out palps) / width (at widest position) of the labial prementum: 3.3 / 2.5 (2.3 / 2.0); length / width of the head: 3.1 / 4.2 (2.6 / 3.5); no differences between female and male dimensions detectable.

Main colour of the larva mid-brown, brighter ventral side because of the thinner integument of the sternites and the lucent-white lateroventral, abdominal gill appendages.

Head at its widest part slightly broader than thorax, length-width ratio of head ~ ¾ on average; external, posterior angle of cephalic lobe rounded (Fig. 1a); on the upper side of the head with 13 symmetrically arranged bare spots visible (Fig. 2); clypeus also free of setae, as well as proximal half of labrum; remaining surface of head covered with scale-like setae; fringy incised integumental border close to compound eyes, in ventral view; each fringe represents the stem of a short seta.

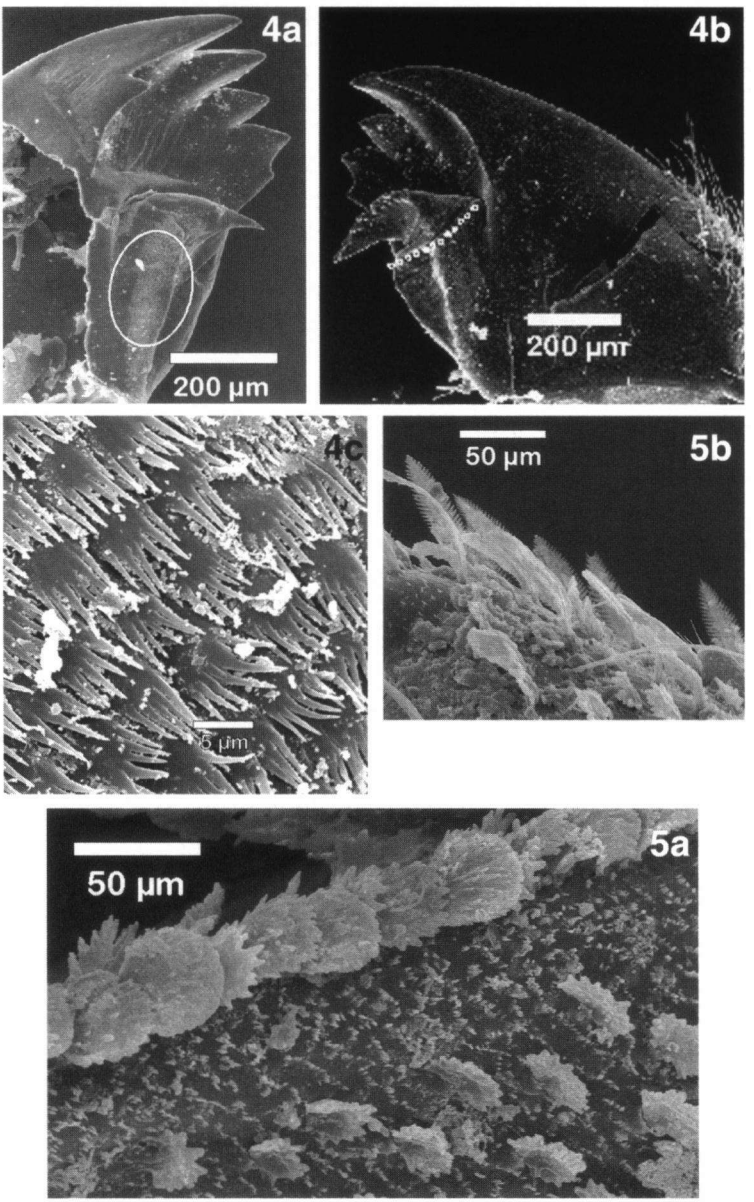
Antenna 7-segmented, length of the segments decreasing from pedicel to 7th segment, except for the second segment after the scape, which is insignificantly longer than the first; joints 3–7 significantly thinner than the first two, no hypertrophied pedicel; also hairiness of the antennae decreases from segment 1–7; first two segments after the scape internally setae with slightly longer than on the remaining antenna surface; interior side of pedicel bearing a group of noticeably longer setae, presumably with sensory function, near the distal margin of the segment.

Articulation of labial prementum reaches prothoracical coxae, length-width ratio of labium (with closed palps, at widest position) approx. 3/2 (Fig. 1b); distal margin of prementum with small, rounded teeth of varying size and with truncated setae between them; the small teeth bearing 1-3 transverse bands, depending on the size of the tooth; median cleft at distal margin of prementum very slight (only around 0.36 % of total premental length, angle: 30°); alternating corrugation proximad from the cleft, visible from dorsal view; spike-like teeth, situated on a slight embossment below the distal margin of the prementum on both sides of the cleft (Fig. 3); no conspicuous setae on dorsal side of prementum, only about ten very small and tiny, symmetrically arranged setae visible from dorsal view; internal edges of palps very finely denticulated; external claw movable; distal margins of palps with 3 pointed teeth, each curved inward; inner tooth more strongly curved, giving the impression of being rounded and shorter; mid tooth longer than the other two.

Maxillae biramous and covered with long, thin setae, presumably with sensory function; palpus absent.

Mandibles uniramous, but with one large, movable tooth, standing out from the remaining teeth, facing ventral side of head (Figs 4a-b); external side of mandibles with presumably sensory hairs; dorsal base of the isolated tooth with a rasp-like area, covered with small, jagged teeth (Fig. 4c).

Thorax broader than abdomen, but narrower than head at its widest part; surfaces of visible parts of thorax widely covered with scale-like setae, but with



Figs 4-5. *Polythore spaeteri*, larval structural features: (4) mandibles: (a) left mandible, internal view (white ellipse marks the rasp-like area); (b) right mandible, internal view (movable site of the inner tooth shown by dotted line); (c) close-up of the rasp-like area, showing small jagged teeth; – (5) surface of tibiae: (a) alternating jagged and rounded flattened setae on the carina; (b) feather-shaped setae, ventral, on distal part of tibia.

crescent-shaped bare areas on each lateral side of the pronotum. Mesothoracical episternum separated by transverse suture; two small bumps on the most lateral points, where the anterior and the posterior halves of the episternum touch each other; spiracle at anterior margin of mesothorax visible more clearly than at episternal plates of metathorax.

Hindwing pads reach beyond abdominal segment 10 (reach posterior margin of segment 3 on average); fore wing pads reach beyond segment 10 (reach posterior margin of segment 4 on average).

Legs decreasing in length from the first to the third pair; tibiae a little longer than corresponding femora; femora laterally compressed, with two ventral and one dorsal carina; tibiae with two ventral and two dorsal carinae, which contrast with the remaining surfaces of the femora by embossment and darker colour. Femora and tibiae covered with scale-like, jagged setae, carinae of tibiae covered with alternating rounded and jagged setae (Fig. 5a); ventral transition between tibiae and tarsi with successive formation of strong, feather-shaped setae (Fig. 5b). Tarsi three-jointed; pretarsi bearing simple claws with expanded bases, and an empodium appearing between the claws and originating inside the praetarsus (Figs 6a-b); tarsi with long, thin setae with presumably sensoric function, especially on the praetarsus (Fig. 6b).

A b d o m i n a l tergites 2-9 with median, slight caudad thorns, whereas thorn only hinted on segment 1, lacking on 10. Posterior margin of each segment appearing ciliated with small setae, posterior margin of 10 with median incision allowing vertical movement of the median caudal appendix (= epiproct), which is originates underneath; depth of incision equals half the length of 10th segment, width corresponds to width of base of a tergal thorn. Caudal appendages (= procts) saccoid and petiolated; length of epiproct on average about 26% of total body length, length of lateral caudal appendages (= paraprocts) on average about 35%; epiproct with bilateral symmetry, with the shape of an inverted triangle; caudal edge of epiproct ending in three equidistant pointed protuberances, mid protuberance is longest; one more protuberance, directed caudad, on each lateral side of epiproct, near the dorsal side, at mid-length of epiproct (Fig. 8c). Shape of paraprocts approximately cylindric, with one protuberance at the caudal end, three further protuberances distributed in distal half of paraproct (Fig. 8c). Thus, the resulting protuberance "formula" is 4(lateral)–5(median)–4(lateral). Surface of epiproct widely covered with very flat scale-like setae that are directed caudad (Fig. 8b), only ventral edge with elongate, capillary setae; surface of paraprocts differs from that of epiproct by presenting a wider area with long capillary setae which increase in length caudad and are condensed to a brush at the caudal end (fig. 8a). Both, the epiproct and the paraprocts, show smaller and larger bare areas without setae; larger bare area of epiproct on lateral sides restricted to proximal 2/3 of the length and to proximal 2/3 of paraproct height; smaller bare area restricted to periphery of petiolated base of epiproct (Fig. 8c);

Table IV

Comparison of differing characters between previously described polythorid larvae and the presumptive larva of *P. spaeteri* ; where comparison is not possible (e.g. due to lack of information or imprecise illustrations), cells contain interrupted horizontal black lines; 1 p. u. i.: presumptive ultimate instar

Species Character	*Cora chirripa*	*Cora cyane*	*Cora marina meridana*	*Euthore fastigiata*	*Chalcothore montgomeryi*	*Chalcopteryx rutilans*	*Polythore spaeteri*
Total body length of final instar exuviae in mm	18.5	13. 5	19.5	~20	~11.5	~13. 5	(p. u. i. larva: up to ~15.0)
Posterior angle of head side lobes	-------	angular	rounded	rounded	well pronounced angles	angular	rounded
Length of sec. antennal joint / length of pedicel	1.2	-------	-------	-------	-------	2.3	~1
Seta arrangement on the first two antennal joints beyound the scape	longer setae on the inner side of both joints, shorter on the second joint	constant, longer setae-length on both joints	constant, short setae-length on both joints	-------	three longer and three shorter setae on the first joint, single seta basal and midway on the second joint	longer setae on the inner side of the first joint and shorter setae on the inner side of the second joint	longer setae on the inner side of both joints, shorter on the second joint, isolated on the first joint
Abdominal segment reached by wingpads	forewing: IV to VI; hindwing: V to VII	forewing: IV; hind-wing: V	forewing: IV; hind-wing: V	hindwing: VI	hindwing: V	forewing: III; hindwing: IV	(p.u.i. larva, forewing: to beyond X; hindwing: to beyond X)
Presence of empodium	yes	yes	yes	yes	yes	no	yes
Presence of tongue-shaped extension on gill -base	no	yes	no	no	no	no	no
"Formula" of protuberances	4-5-4	6-6-6	5-6-5	4-5-4	4-5-4	3-3-3	4-5-4
Protuberance size	very short	-------	short, very similar to *P. spaeteri*, but more pointed	shorter than *Ch. montgomeryi*, longer than *C. chirripa*	very long (approx. half the length of the appendage), almost bare	-------	short, similar to *C. chirripa*

larger bare area of the paraprocts spread over nearly whole width of ventral side and almost 3/4 of length of paraproct length (Fig. 8c); larger bare area bordered by longer setae anteriorly, setae progressively change to flattened shape toward posterior; smaller bare area of paraprocts restricted to periphery of petiolated base of the appendage.

Additional caudal appendages, the cerci, present, corresponding to appendices superiores of the adult.

Tubular, tapering gills on ventral side of the segments 2-7, near the lateral margins (Fig. 7); completely extended gills reach almost half the length of the abdomen, but under natural conditions the proximal 3/4 of the length are adducted to the abdomen and slightly contorted, while the distal 1/4 is strongly turned outwards; at the respective "knee" each gill is most strongly tapered; in the distal 3/4 up to eight segmentation-like constrictions with approximate regularity; external, proximal half of gill-like appendages covered with scale-like setae, remaining gill surface bare; on some specimens, injuries to the gill integument allowed insight into internal structures such as basal dorsoventral muscles and longitudinally proceeding tracheae.

Female gonapophyses and male gonapophyses, the latter hardly reach beyond posterior border of segment 9.

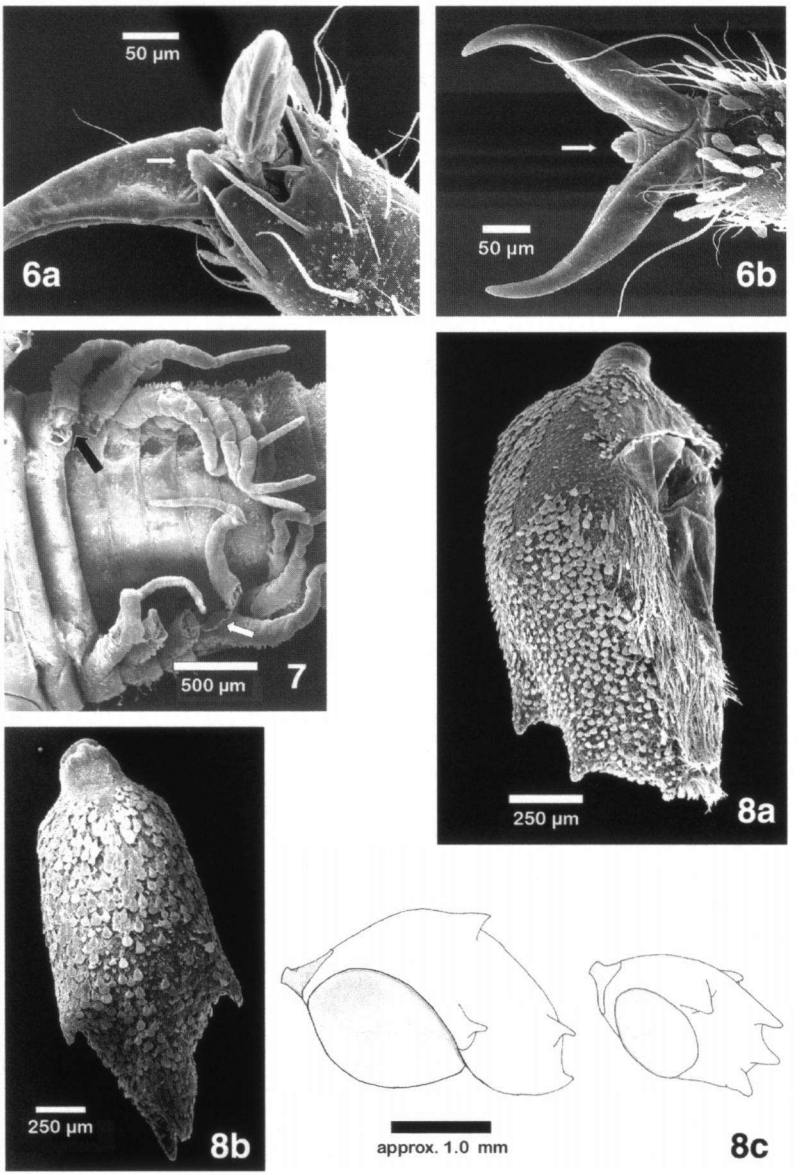
DISCUSSION

MOLECULAR ANALYSIS

Mitochondrial markers have proved useful to the study of genetic variation and species limits in insects (e. g. SPERLING & HICKEY, 1995; LANDRY et al. 1999). The cytochrome oxidase subunit I (= COI) gene possibly has the potential being a general tool for assignment of newly analysed taxa at the species level, as HEBERT et al. (2003b) demonstrated by generation of extensive, "barcode"-like COI profiles.

As the results of the present study show clearly, there is no conspicuous sequence divergence between the larval and the imaginal COI sequences. The comparitively higher p-distance among the imaginal sequences can be explained by the wider collecting range of the specimens. In contrast, the larvae derive from a single population; theoretically they could even derive from the same egg batch.

The mitochondrial COI gene is the most conserved gene concerning the evolution of amino acids (SIMON et al., 1994). The substitution rate on the third-codon positions is three times higher in the COI gene than in the 12S or 16S rRNA genes, for example (HEBERT et al., 2003b). Phylogeographical examinations have shown that intraspecific COI-sequence divergence values normally range between 1-2%, mostly they do not reach the 1% value. In contrast, interspecific values of nearly 100% of the examined congeneric species-pairs resulted in values



Figs 6-8. *Polythore spaeteri*, larval structural features: (6) (a-b) ventral and dorsal view of distal end of pretarsus (arrow points at empodium); – (7) ventral view of abdominal segments 2-7, bearing lateroventral gills, injured sites allow insight into internal structures (black arrow points to part of a basal, dorsoventral gill muscle; white arrow points to a partly decoiled trachea emerging from the gill base); – (8) procts: (a) paraproct, dorsal view; (b) epiproct, dorsal view; (c) paraproct (left) ventrolateral view and epiproct, lateral view (bare areas are shown in grey).

beyond 2% (HEBERT et al., 2003a). Compared to interspecific p-distance values of other closely related species, the p-distance values between imaginal and larval *P. spaeteri* specimens are extremely low. This leaves rather little doubt about the conspecificity of *P. spaeteri* imagines and the larvae described here.

P-distance values to the reference species are much higher, as expected.

COMPARISON OF CURRENTLY AVAILABLE DESCRIPTIONS OF POLYTHORID LARVAE WITH THE DIAGNOSIS OF THE PRESUMPTIVE *P. SPAETERI* LARVA

The average body length of larvae declared in published descriptions differs to some extent. However, due to the lack of final instar exuviae of *P. spaeteri*, direct comparison is not possible. The same applies to wing-pad length in relation to the abdominal segments. The corresponding values of *P. spaeteri* stated in tab. 4 only give an indication for those of the ultimate instar. It is also not clear whether the remarkable maximum length of the wing pads in *P. spaeteri* is only an artefact, possibly effected by compression of the abdomen.

The side lobes of the head show dorsal, posterior "angles", which vary between a rounded and truly angular form. In all described polythorid larvae the second antennal segment after the scape is the longest, but the length ratio between the second antennal segment after the scape and the pedicel differs from species to species. Another differing character is the arrangement of the presumably sensory setae on the inner side of the pedicel and the following antennal segment. This arrangement varies from short hairs, similar on both antennal joints, to hairs strongly differing in length between the two joints.

The only notable difference concerning the legs is the presence or absence of the empodium, which was referred to as "empodium-like structure" by Calvert (1911) and the following descriptions of polythorid larvae. The SEM exposures of the pretarsus of the *P. spaeteri* larva show that there is no doubt about the structure being a true empodium.

No comparison is possible concerning the feather-shaped setae on parts of the tibiae and tarsi, because earlier descriptions of polythorid larvae do not mention this character. However, MacNEILL (1967) described in a comparative morphological study, comprising several zygopteroid and anisopteroid larvae, so-called "pedal-combs" on tibia and tarsus of the larvae. Via light-microscopy he was able to divide most of these combs into groups with "furcate", "pectinate" or "furco-pectinate" shape. As representatives of the Calopterygidae he inspected *Calopteryx splendens* and *C. virgo* larvae which possess only flat, pectinate combs, mainly at the ventral transition between tibia and tarsus. The inspected calopterygid larvae feature a complete lack of furcate "combs". Concerning this character they apparently take an exceptional position. A comparison with the illustrations of the mentioned "combs" in the calopterygid larvae (MacNEILL, 1967) shows

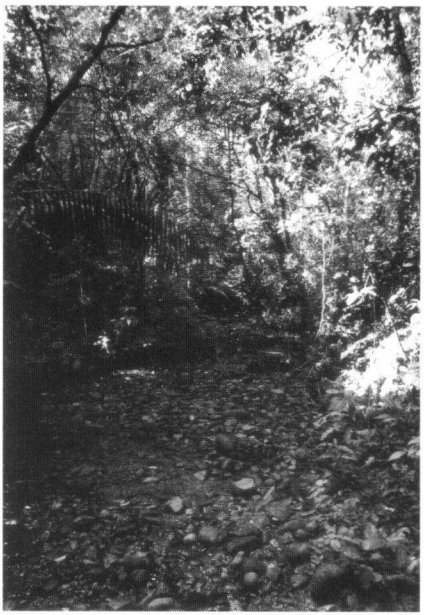


Fig. 9. *Polythore spaeteri*, larval habitat.

that their shape seems to be very similar to that of the feather-shaped setae of larval *P. spaeteri*. Also NOVELO-GUTIÉRREZ & GONZÁLES-SORIANO (2004) described "four-branched setae on distal end of protibia" in a larva of *Dythemis maya* Calvert, which feature clearly the furcate type after MACNEILL's (1967) idea. Further investigations are necessary to evaluate the comparability and diagnostic value of this character at the generic level.

An apparent difference is the occurrence of a tongue-shaped extension on the base of the lateral gills, described for *Cora cyane*. No other description contains the characterisation of a comparable structure, but there are some undescribed polythorid larvae from Venezuela and Ecuador with lateral gills featuring strongly thickened basal parts which are armed with thorn-like setae to some extent (De Marmels, pers. comm., 2004; authors' observations). Among the polythorid larvae so far described the illustration of *Chalcothore montgomeryi* also shows basal gill parts differing from the remaining, membranous gill part, but apparently without distinct extensions (DE MARMELS, 1988).

The caudal appendages present the character with the perhaps highest diagnostic value (DE MARMELS, 1992): the varying number of protuberances on the paraprocts and epiproct, presented in the following tab. 4 as a "protuberance formula" (for details see description). Larvae featuring the same protuberance formula differ in protuberance size to some extent, while the basic shape of the caudal appendages apparently does not vary among the already described larvae.

Table IV shows the explicit differences in the characters named above for the previously polythorid larvae described and the larva of *P. spaeteri*.

According to the character state differences in Table IV, *P. spaeteri*, *E. fastigiata meridana* and *C. chirripa* show the highest degree of overlap, while *E. fastigiata meridana*, *C. cyane*, *C. chirripa* and *Ch. rutilans* only agree in one of the listed characters, respectively. Remarkable is the fact that there is no character state shared collectively by members of the genus *Cora*. Especially *C. cyane* seems to be quite outstanding because of its high number of protuberances on the procts and its tongue-shaped extension on the gill basis. If a similar quantity of divergence can be recognised between *Cora* imagines, the genus definition of *Cora*

may have to be re-evaluated. Further statements on the generic level cannot be made until more polythorid larvae are described. The larva of *P. spaeteri* cannot be differentiated from the other known polythorid larvae by a single feature, but by a combination of characters.

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