

***Nicrophorus mexicanus* (Coleoptera: Silphidae: Nicrophorinae): larval morphology and phylogenetic considerations on the *N. investigator* group**

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Abstract. The authors describe and illustrate in detail the three larval stages of *Nicrophorus mexicanus* Matthews, 1888 (Coleoptera: Silphidae: Nicrophorinae), a common mexican and central-american carrion beetle. A study of the taxonomic value of larval characters, as well as the morpho-anatomical characters of adults, enables the authors to discuss the phylogenetic relationships among the species of the *N. investigator* group, which includes *N. mexicanus*. The species seems to be closely related to *N. investigator* Zetterstedt, 1824 and *N. nigrita* Mannerheim, 1843, while the remaining two species classified in the group (*N. hybridus* Hatch & Angell, 1925, and *N. tomentosus* Weber, 1801) appear to be more primitive. Results partly confirm phylogenetic hypotheses proposed by other authors in previous papers.

Larval morphology, phylogeny, Coleoptera, Silphidae, *Nicrophorus*

INTRODUCTION

Nicrophorus mexicanus Matthews, 1888 is a large necrophagous beetle, active all year round, distributed from southwestern United States to Guatemala and El Salvador (Peck & Anderson 1985).

Building on the classic studies of Hatch (1927), Pukowski (1933, 1934) and Balduf (1935), more recent researches have focussed on the immature stages of *Nicrophorus* Fabricius, 1801 species from both a morphological (Byzova 1964; Klausnitzer & Zerche 1978; Anderson 1982; Peck & Anderson 1985; Růžička 1992) and an ethological (Roussel 1964a, b; Milne & Milne 1944, 1976; Halffter et al. 1983; see also the papers quoted in Huerta et al. 1992) perspective.

The three larval instars of *Nicrophorus mexicanus* are described in this paper in detail, with a view to reexamine the phylogenetic relationships of the species. Our results are compared with those of Peck & Anderson (1985) who placed five species of *Nicrophorus* (*N. hybridus* Hatch & Angell, 1925, *N. investigator* Zetterstedt, 1824, *N. mexicanus*, *N. nigrita* Mannerheim, 1843 and *N. tomentosus* Weber, 1801) – mainly from Latin America – together in the *N. investigator* group. Their study included five characters taken from larval morphoanatomy, which formed the basis of a phylogenetic hypothesis.

MATERIALS AND METHODS

Twelve individuals (5 females and 7 males) from the Chapultepec Wood, Mexico City, Mexico, were included in this study. The individuals were collected during summer 1991 with pitfall traps baited with meat. Five pairs were secluded and reared in controlled conditions in suitable terraria, 30 cm in diameter at the mouth, 42 cm high, 3/4 full of sieved sandy soil, at the Department of Animal Biology, University of Turin, Italy. Ground beef was used as food. Terraria were opened and observed at irregular times depending on the activity of each pair. The five pairs regularly bred so that 6 first instar (L_1), 8 second instar (L_2), and 12 third instar (L_3) larvae were obtained (no pupa was sacrificed, so that a description of this stage is not provided). On average the first larval instar took 2 days to develop, the second instar 4 days, the third instar 6 days, and the pupa 25 days.

These data are in keeping with the observations of Halffter et al. (1983) for species raised under laboratory conditions. Larvae were dropped into boiling water for about three minutes; subsequently they were fixed in Bouin solution and transferred after two days into 70% ethyl alcohol. Some were dissected and mouth parts, antennae, and legs were examined first in glycerine, and then in Canada balsam under the microscope.

DESCRIPTION

Larva eruciform and elongate (Fig. 1). Dorsoventrally depressed. Body whitish, third stage more brownish, with the head, strongly reduced ventral sclerites, regions surrounding the spiracles, urogomphi (Figs 1, 18, 38) and legs more sclerotized. Average body length: L_3 27.8 mm, L_2 18.8 mm, L_1 5.2 mm.

HEAD (Figs 11, 12, 13, 29). Prognathous, dorsoventrally depressed. Average width: L_3 2.2 mm, L_2 2.1 mm, L_1 1.0 mm; average length: L_3 1.8 mm, L_2 1.5 mm; L_1 0.9 mm. Epicranial suture short, marked, straight. Frontal suture V-shaped, with branches very divergent, turned laterally near the fovea, and less conspicuous near the insertion of antennae. Frons gently convex and medially more sclerotized. Epistomal suture manifest only laterally. Foveas well marked.

CLYPEUS (Figs 23, 42). Pentagonal in form, separated from labrum by a large unsclerotized band, with about six sensilla and grouped together with three pairs of setae. L_2 and L_1 clypeus less sclerotized than in L_3 .

LABRUM. L_1 : transverse, slightly convex apically, pentagonal in form, on the whole not very sclerotized. Central part clearly transverse, without a semicircular area. Anteriorly four strong setae, posteriorly six more, less robust, and two sensilla. L_2 and L_3 with anterior central part strongly sclerotized and directed apically, and two unsclerotized bands laterally. Anterolateral angles well-marked. Setae like in L_1 . L_2 : anterior margin clearly concave in the middle, very sclerotized laterally, semicircular, raised apically with two teeth.

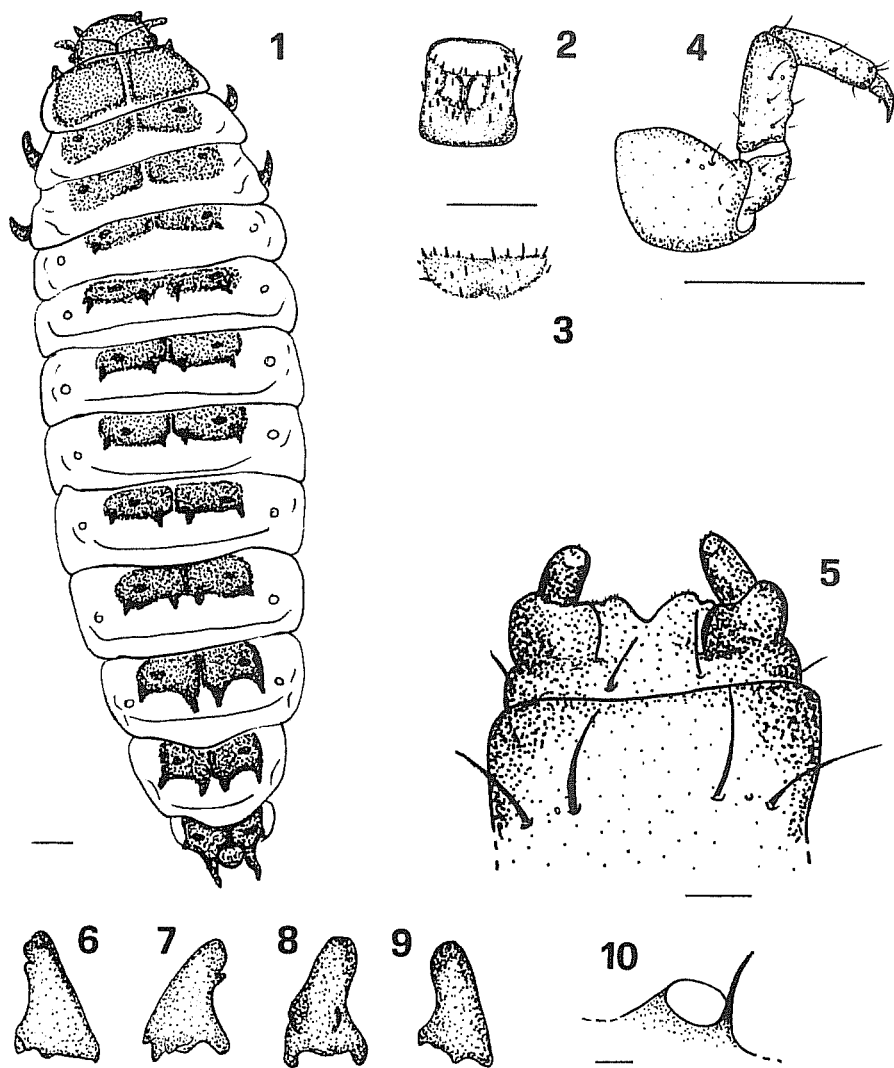
EPIPHARYNX. Two longitudinal bands of dense microtrichia directed centrally; medial part glabrous. L_1 : anterior medial part poorly developed, bearing centrally the first porous area, and laterally two strong, conical setae. L_2 (Fig. 25) and L_3 : anteromedial concavity more evident. In all instars the first porous area divided by a longitudinal septum and bearing at least three-four pairs of sensilla. Posterior to these the second porous area, pair, with a triad of sensilla. Latero-anteriorly two pairs of conical setae. At the base, between the two longitudinal bands, an irregular, transverse row of variable numbers of sensilla (quinqueporous area) and laterally and posteriorly two groups of five sensilla, in clusters (Fig. 26).

MANDIBLES. Strongly sclerotized. Coarsely pyramid-like, without molar areas. L_3 with spatulate scissorial area in the left mandible (Figs 8, 9), slightly notched, making a small tooth, in the right one (Figs 6, 7). In L_2 mandibles bearing a scissorial area clearly divided by a big median tooth in two parts, each provided with five small teeth. Four hairs dorsolaterally in the right mandible (Figs 21, 22), only one in the left (Figs 19, 20). L_1 : mandibles more slender and shorter than in L_2 and L_3 . Scissorial areas slightly notched. Externally two hairs, laterally two sensilla (Figs 34, 35).

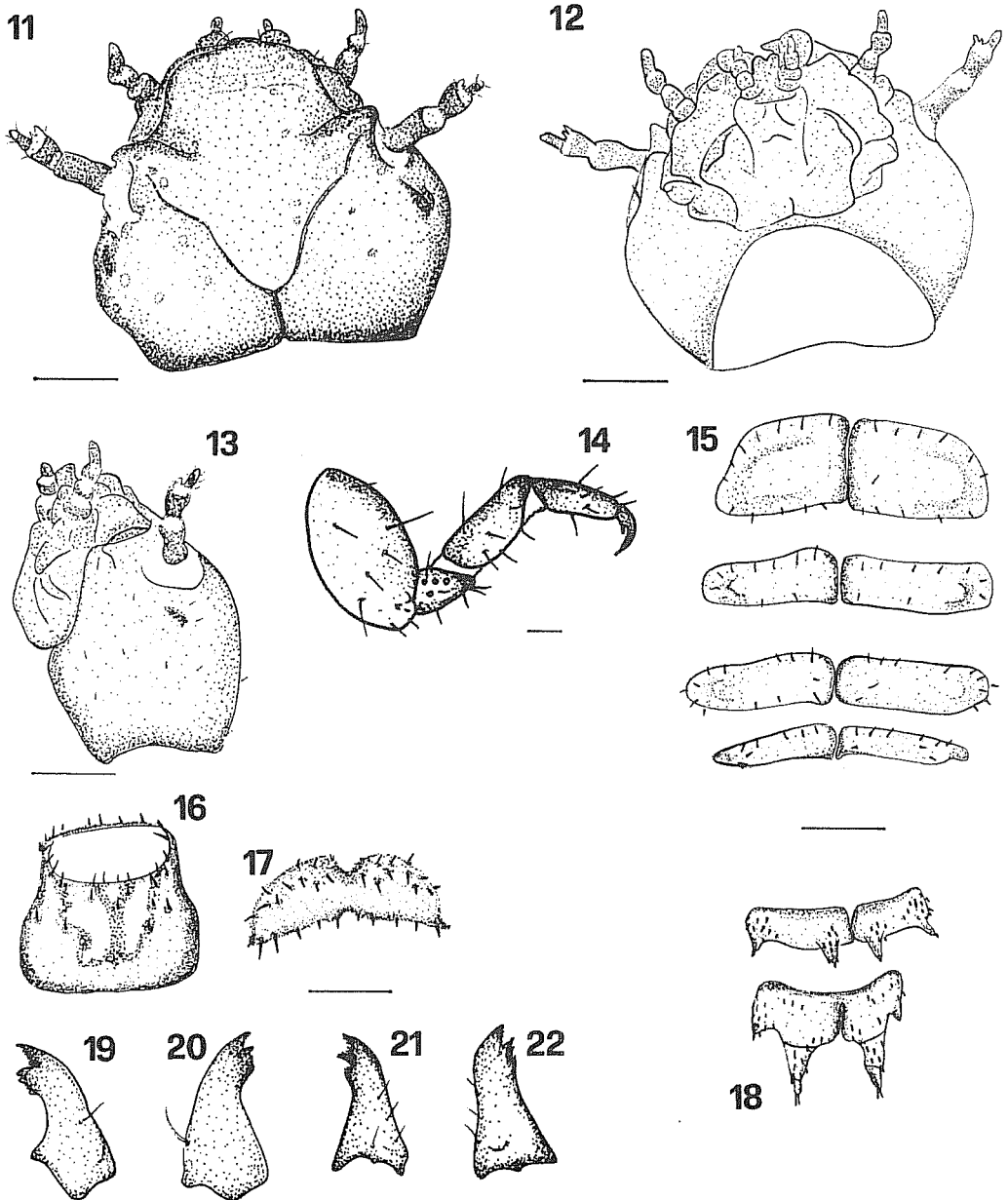
MAXILLAE (Figs 28, 39). The labio-maxillary complex appears more sclerotized in L_3 than in L_1 and L_2 . Cardo pyramidal with apex blunt and a posterior seta. Stipes conical, truncated, robust with five primary setae. Palpiger cylindrical, more or less equivalent in size to the first segment of palpi, separated from the maxilla by a small sclerotized area. Palpiger lacking in narrow sclerotized belt and bearing one seta.

Maxillary palpi three-segmented; segments I and II cylindrical, segment III conical. Segment I bearing two flat sensilla; II with two setae and one flat sensillum; III with apical area bearing dense microsensilla and two small setae, one distal and one proximal.

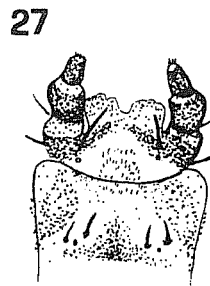
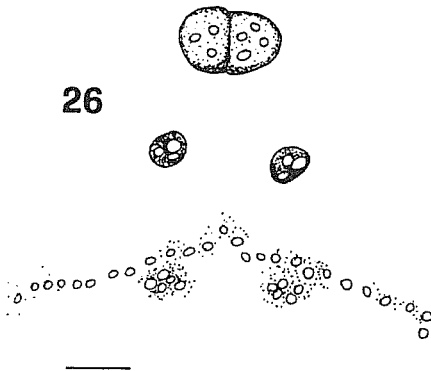
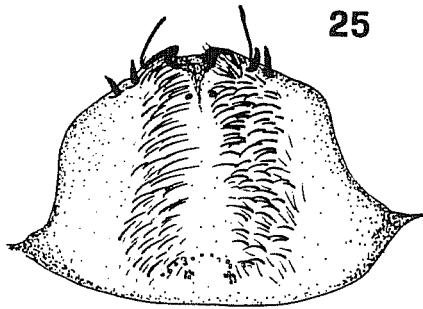
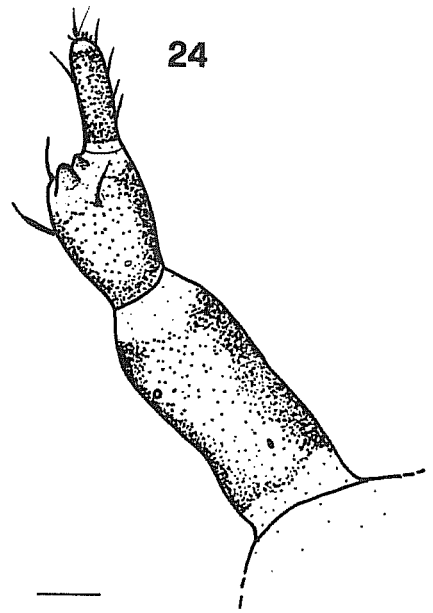
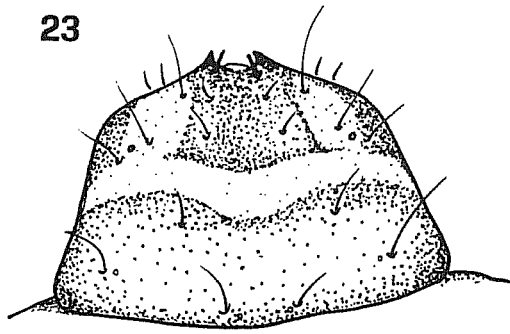
Mala bearing one strong seta inserted on the external edge with a lateral process at the base, small in L_1 , stronger in L_2 and L_3 . Dorsal process of mala not always clearly visible. Ventral lobe usually small. Inner basal angle sclerotized and pronounced, bearing an area densely covered with microtrichia. Base of galea less sclerotized than mala. Distal area with a transverse row of dense, strong setae. Lacinia narrow and oblong, clearly bilobed. Inner lobe narrow and elongate



Figs 1-10. *Nicrophorus mexicanus* Matthews, L_3 . 1 - larva *in toto*, dorsal view, 2 - X abdominal segment, ventral view, 3 - IX abdominal segment, ventral view, 4 - left methathoracic leg, 5 - labium, dorsal view, 6 - right mandible, dorsal view, 7 - right mandible, ventral view, 8 - left mandible, dorsal view, 9 - left mandible, ventral view, 10 - anular spiracle of mesothoracic epipleuron. Line bars: 1 mm, except figs 5 and 10 (0.1 mm).

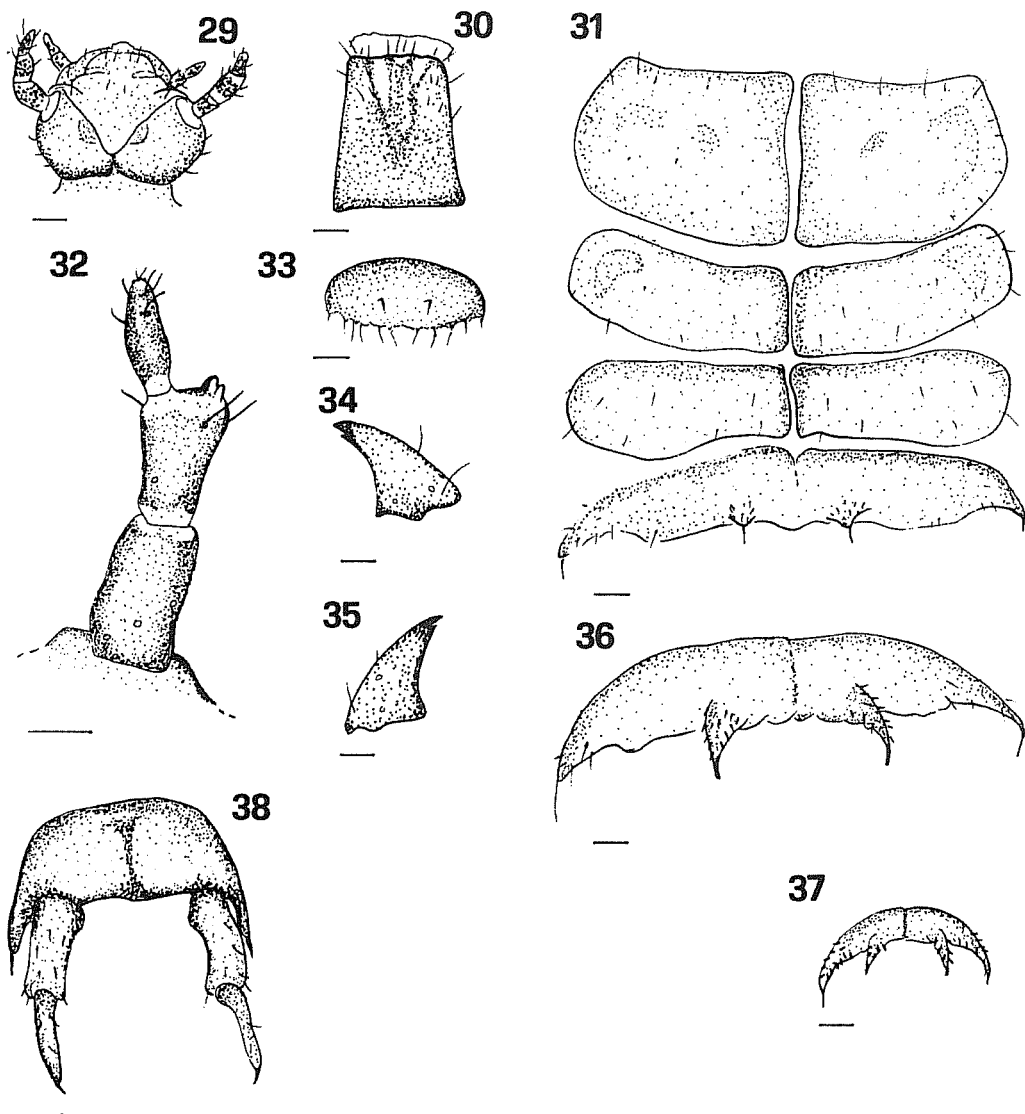


Figs 11–22. *Nicrophorus mexicanus* Matthews, L₂. 11 – head, dorsal view, 12 – head, ventral view, 13 – head lateral view, 14 – left metathoracic leg, 15 – three thoracic and first abdominal dorsal scutal sclerites, 16 – X abdominal segment, ventral view, 17 – IX abdominal segment, ventral view, 18 – VIII and IX abdominal segments, 19 – left mandible, dorsal view, 20 – left mandible, ventral view, 21 – right mandible, ventral view, 22 – right mandible, dorsal view. Line bars: figs 11–13, 16, 17, 19–21: 0.5 mm, fig. 14: 0.1 mm, figs 15 and 18: 1 mm.



Figs 23–28. *Nicrophorus mexicanus* Matthews, L₁. 23 – clypeus, 24 – antenna, 25 – epipharynx, 26 – basal sensilla of epipharynx, 27 – labium, dorsal view, 28 – maxilla, ventral view. Line bars: 0.1 mm.

with three strong apical teeth and membranous ventral region bearing an area of dense microtrichia. External lobe with five teeth: the tooth close to the galea more developed and stronger. LABIUM (Figs 5, 27, 41). Postmentum truncoconical with three strong setae on each side. Mentum cylindrical, short, with anterior angles rounded, sharp, and two setae on each side separated

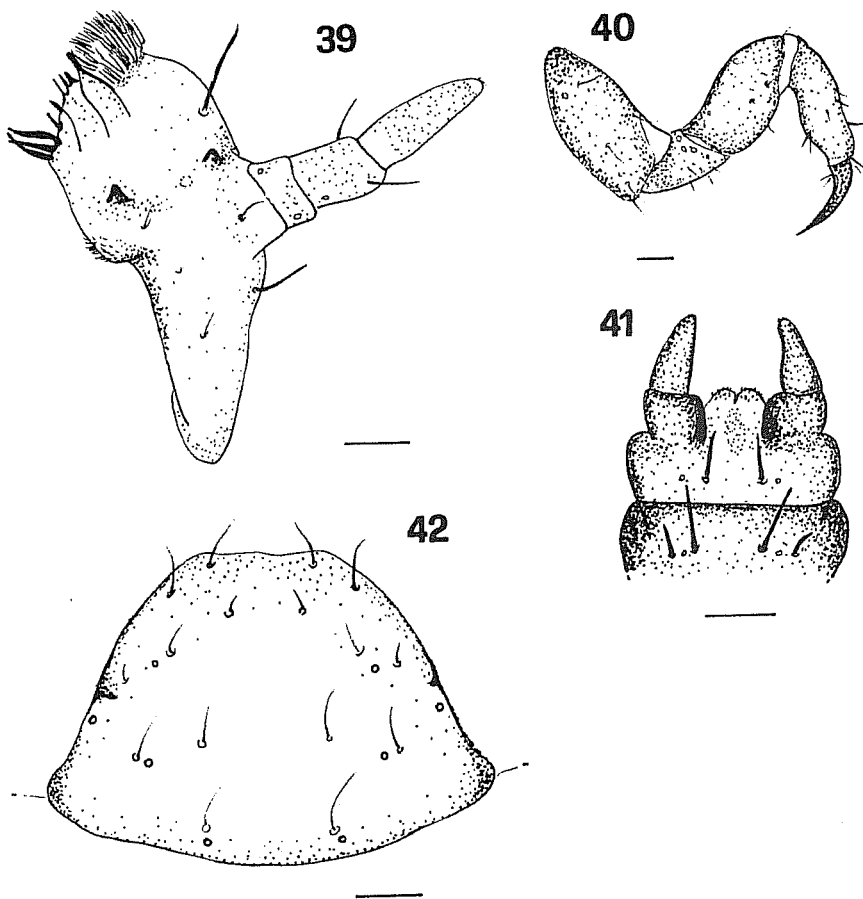


Figs 29-38. *Nicrophorus mexicanus* Matthews, Lj. 29 - head, dorsal view, 30 - X abdominal segment, ventral view, 31 - three thoracical and first abdominal dorsal scutal scleres, 32 - antenna, 33 - IX abdominal segment, ventral view, 34 - right mandible, dorsal view, 35 - right mandible, ventral view, 36 - V abdominal segment, 37 - VIII abdominal segment, 38 - IX abdominal segment. Line bars: 0.1 mm.

by a sensorial pore. Praementum transverse, more sclerotized at hind angles that bear one strong seta, and mesally convergent along two distal bands, bearing centrally a pore and a strong seta. Labial palpi two-segmented. Segment I transverse. Ventral surface sclerotized. Segments II and III more elongate, apically unsclerotized and bearing small sensilla. L_1 : ligula medially joined, L_2 and L_3 : widely disjoined with thickened and short setae apically.

ANTENNAE (Figs 24, 32). Three-segmented. First segment cylindrical, with at least three sensilla. Second segment swollen and less sclerotized apically, with two proximal sensilla; two more apically, conical and more noticeable, of unequal size; and three setae subapically. Width of the third segment about half that of the second, inserted asymmetrically, bearing 4-5 sparse setae and, apically, two more minute setae and two small microsensilla.

THORAX. Dorsoventrally flattened. Dorsal scutal sclerites strongly sclerotized, medially interrupted. Pronotal sclerite more developed than the other two. Marginal regions with small, primary, reddish setae. L_3 : antero-lateral regions with muscle attachments visible as darker spots, not evident in L_2 and L_1 . Posterior edge of scutum bearing no lobes or spines (Figs 15, 31).



Figs 39-42. *Nicrophorus mexicanus* Matthews, L_1 . 39 - left maxilla, ventral view, 40 - left methathoracic leg, 41 - labium, dorsal view, 42 - clypeus. Line bars: 0.1 mm.

Tab. 1. Characters and character states used to build the phylogenetic tree

	Character	plesiomorphic state (0)	apomorphic state (1)
1	Overwintering stage	adult	prepupa
2	Adult metasternum	lacking bald spot	with bald spot immediately posterior to mesocoxae
3	Adult pronotum	subquadrate to cordate	quadrate
4	Adult metasternal pubescence	yellow	brown
5	Apical part of venter of tenth segment, L ₃	sclerotized	unsclerotized
6	Bases of larval labial palpi	widely separated	narrowly separated
7	Suture at base of urogomphus, L ₃	complete	absent

Regions of notum close to unsclerotized lateral alar lobe largest and rounded on prothorax. On meso- and metathorax shorter and wedge-shaped, dividing epipleurum into anterior and posterior parts. Anterior epipleuron of mesothorax showing an anular spiracle; a medium size hair inserted on lateral-inner edge of spiracle (Fig. 10); metathorax only with small sclerotized area corresponding to vestigial spiracle on lateral sides.

Pleuron with sclerotized episternum. Proepimeron not sclerotized, meso- and metaepimeron with triangular sclerotized area. On the ventral thoracic surface, the prothoracic praesternum transverse, wide, swollen and with a sclerotized area laterally. L₂: prothoracic praesternum not swollen centrally, and on the whole little sclerotized. Meso- and metathoracic praesterna particularly developed laterally and apparently interrupted medially. Basisterna and sternella of all segments bearing about 40 short, robust, reddish-brown setae. L₂: prothoracic basisternum more flattened medially than in L₃.

ABDOMEN. Ten-segmented. Segments I–V short, dorsoventrally flattened (Fig. 36). Segments VI–VIII gradually longer and narrower, IX considerably modified (Figs 3, 17, 33), X converted into an anal tube (Figs 2, 16, 30). L₂: segments I–IV longer and wider than segments V, VI and VII. Segment VIII clearly longer and narrower (Figs 18, 37).

A transverse suture dividing the notum dorsally into anterior scutal sclerite (praescutum and scutum fused together) and posterior short unsclerotized scutellum. Scutal sclerite about as long as that of metathorax, but distinctly narrower, with paler medial line. L₁: sclerite reaching half the length of the notum, whereas in L₂ and L₃ it reaches the first third only. The posterior margin

Tab. 2. Data matrix

	1	2	3	4	5	6	7
<i>N. fossor</i>	1	0	0	0	0	0	1
<i>N. investigator</i>	1	0	1	0	0	0	0
<i>N. nigrita</i>	1	0	1	1	0	0	0
<i>N. tomentosus</i>	1	1	0	0	1	0	0
<i>N. hybridus</i>	1	1	0	0	1	0	0
<i>N. mexicanus</i>	1	0	1	1	0	1	0
<i>P. morio</i> (outgroup)	0	0	0	0	1	0	0

extended to form one pair each of lateral and dorsal lobes or spines. Spines tubercle-shaped in segments I–II–III; elongate and pointed in remaining ones. Dorsal and lateral spines equivalent, except in segment IX where the dorsal one is clearly longer. Spines bearing a large number of short and dumpy setae. Pleural lobe with a number of small setae and lacking in oval sclerotized areas.

Laterally segments I–VIII bearing one anular, sclerotized spiracle on each side, smaller than mesothoracic ones.

Basisternum with rhomboidal, small, central sclerite, increasing in size from segments II to VIII. L₂: basisternum showing an anterior, vestigial line, whole in the I and II segments, mesally indistinct in remaining ones.

LEGS (Figs 4, 14, 40). Relatively short, heavily sclerotized, except for an unsclerotized medial coxal area. Coxa short, wide and tronco-conical. Trochanter short. Femur and tibiotarsus subequal in length, subcylindrical; the last one narrower. Praetarsus slender, narrow, with two small ventral setae and an arcuate claw. L₂: medial coxal area unsclerotized; tibiotarsus bearing two setae and a crown of sensilla apically.

Identification key of larval instars

To facilitate the identification of the larval instars of *N. mexicanus*, we propose a key based on morphoanatomical characters. The following structures appear to carry most significance: apex of segment X, urogomphi, mandibles, maxillae, labium and antennae.

- 1 2nd segment of labial palpi twice as long as 1st. 3rd segment of maxillary palpi twice as long as 2nd. Lateral external process of mala minute. Lobes of ligula parallel and with distal edges not notched. Mandibles simply triangular, with apex narrow and slightly notched. Clypeus little sclerotized with anterior edge rectilinear. 1st antennal segment as long as the 2nd. Tibiotarsus just longer than praetarsus. Apex of segment X ventrally sclerotized. Urogomph swollen. L₁
- 1' 2nd segment of labial palpi about as long as the 1st. 3rd segment of maxillary palpi about as long as the 2nd. Lateral external process of mala well-developed. Lobes of ligula clearly distinct with two notches in apical edge. Mandible with apex swollen. Clypeus very sclerotized with anterior margin medially hollowed. 1st antennal segment twice as long as the 2nd. Tibiotarsus twice as long as the praetarsus. Apex of segment X unsclerotized ventrally. 2
- 2 1st segment of labial palpi just wider than the 2nd. Apex of mandible with triangular, small and irregular teeth. Clypeus quite sclerotized except for a median C-shaped area L₂
- 2' 1st segment of labial palpi clearly wider than the 2nd. Apex of mandible frequently rounded through wear. Clypeus very sclerotized and well-developed L₃

DISCUSSION

Knowledge of the preimaginal instars of *N. mexicanus* supplies us with further characters for assessing the systematic position of this species within the *N. investigator* group (sensu Peck & Anderson 1985), in addition to those adult characters that have been reported in the literature.

We generated a ten-character matrix (Tables 1, 2), and analyzed it using both the PAUP 3.0b (Swofford 1989) and Macclade 3.0 (Maddison & Maddison 1992) packages for inferring phylogenies. Character polarities were inferred by outgroup comparison with the only other genus known for the subfamily Nicrophorinae, i. e. the Asiatic *Ptomascopus* Kraatz, commonly regarded as primitive. Following cited literature, we took into account the species *P. morio* Kraatz. The states of characters 6, 7, 8 and 9 were inconsistent among the three larval instars (in keeping with the findings of Růžička (1992) for the palearctic species) and therefore were analyzed separately. For larval instars unavailable to us we referred to the data published by Anderson (1982) and Peck & Anderson (1985). The polarity of characters 6 and 7 was based on the outgroup criterion, used by Peck & Anderson (1985). Our data do not confirm the polarity given by

Růžička (1992), who employed an ontogenetic criterion. Characters were considered as irreversible and the outgroup was used to root the tree. Characters 1, 9, and 10 proved to be uninformative, the last two being autoapomorphies of *N. mexicanus*. The results of our phylogenetic analysis (Fig. 43) allowed us to formulate the following hypotheses:

- according to Peck & Anderson (1985), *N. tomentosus* and *N. hybridus* (both distributed in the Nearctic region) are sister taxa, and they are probably the most primitive species within the *N. investigator* group.
- the three remaining species of the group appear to be more derived and closely related.

Nevertheless, our data are not sufficient to analyze relationships among *N. nigrita*, *N. investigator* and *N. mexicanus*, and we will need further information, both on adult and larval morphology, to resolve the polytomy. At this stage of the research, we cannot confirm the phylogenetic hypothesis of Peck & Anderson (1985) (who did not take into account larval characters of *N. mexicanus*). In fact, our matrix supports three phylogenetic trees of the same length (12) and statistics (CI=0,83, RI=0,80, Rescaled CI=0,67), yielding, after a strict consensus analysis, the unresolved tree shown in Fig. 43.

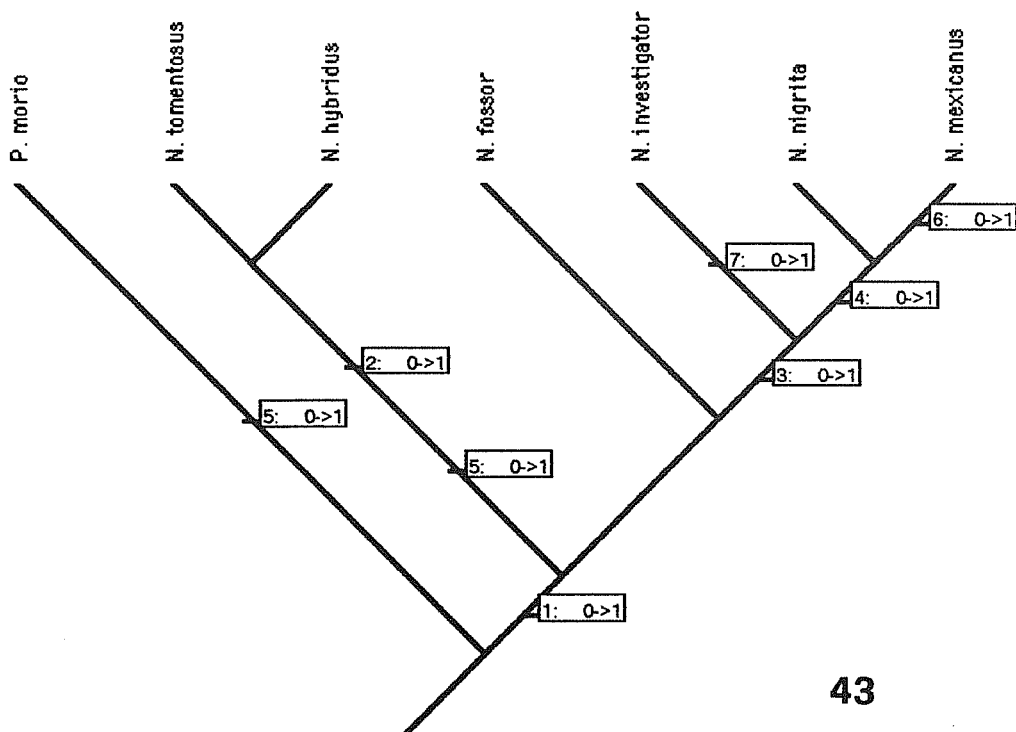


Fig. 43. Hypothetical phylogenetic relationships among species of the *N. investigator* Zetterstedt group.

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