Eur. J. Entomol. **100**: 417–428, 2003
ISSN 1210-5759

Taxonomy of European *Eristalinus* (Diptera: Syrphidae) based on larval morphology and molecular data

CELESTE PÉREZ-BAÑÓN[1*], SANTOS ROJO[1], GUNILLA STÅHLS[2] and M. ANGELES MARCOS-GARCÍA[1]

[1]CIBIO (Centro Iberoamericano de la Biodiversidad), Universidad de Alicante, Alicante E-03080, Spain
[2]Finnish Museum of Natural History, University of Helsinki, P.O. Box 17, FIN-00014 Helsinki, Finland

Key words. Syrphidae, *Eristalinus*, immature stages, morphology, life history, molecular data, Europe

Abstract. The taxonomy of European *Eristalinus* syrphid flies is reviewed. New data on their life histories, biological notes and a key to species using pupal characters are provided. The larvae and puparia of *Eristalinus taeniops* (Wiedemann, 1818) and *Eristalinus megacephalus* (Rossi, 1794) are described for the first time, including new morphological characters of the thoracic respiratory process of all species. The morphology of the male genitalia of *E. megacephalus* is described and compared with that of *E. taeniops*.

The results of our morphological studies of the male genitalia and molecular data (mitochondrial COI and nuclear 28S rDNA) do not support the traditional adult classification based on the patterning on the eyes (fasciate *vs* punctate). We present a phylogeny of the species based on molecular data. The molecular and morphological data indicate that the relationship between some species with punctate eyes and those with fasciate eyes may be closer than with other species with punctate eyes. Moreover the results of the molecular studies support two clades, which does not accord with the traditional arrangement of this group of Syrphidae.

Accordingly we propose that the characters of male genitalia stated by Kanervo in 1938 (but subsequently largely ignored) for arranging the European species of the *Eristalinus-Eristalodes-Lathyrophthalmus* complex, are suitable for classifying these species.

INTRODUCTION

The concept and delimitation of the genus *Eristalis* Latreille, 1804 is variable. According to Thompson (1997), Meigen (1822) restricted the name *Eristalis* to those species defined by two wing characters, a sinuate wing vein R4+5 and a petiolate cell R1. Later, some authors divided *Eristalis*. For the Palaearctic species, Rondani (1845) described the subgenus *Eristalinus* and Mik (1897) described the genera *Eristalodes* and *Lathyrophthalmus*. These three groups were erected based on traditional morphological characters (pattern of pilosity on the arista, the pattern of pigmentation on the eyes, state of eye contiguity in males). Some authors considered these three taxa as valid genera (and different from *Eristalis*) (i.e.: following Sack, 1928–1932), but others treat the 3 groups as subgenera of *Eristalis* (i.e.: Shiraki, 1930). A novel attempt at subdividing *Eristalis* (*sensu* Meigen, 1822) was made by Kanervo (1938), based on characters of the male genitalia, but his work was largely ignored. Thompson (1972) re-examined Kanervo's work, and following Kanervo (1938) divided the Neotropical species into a few groups, also based on male genitalia, but did not consider the above mentioned groups as they are not present in the Neotropical region. Recently, Hippa et al. (2001) reviewed the West Palaearctic species of *Eristalis* (*sensu stricto*) and *Eoseristalis* Kanervo, 1938, but not those in the genus *Eristalinus* (*sensu lato*).

About 75 species of *Lathyrophthalmus*, 13 species of *Eristalodes* and 2 of *Eristalinus* have been described (Thompson, 2002). Most of these species are from the Afrotropical and Oriental regions and only 14 occur in the Palaearctic region (Thompson & Rotheray, 1998). In the Catalogue of Diptera of the Afrotropical region (Smith & Vockeroth, 1980) and the Manual of Palaearctic Diptera (Thompson & Rotheray, 1998), these three groups of species are considered as subgenera of the genus *Eristalinus* Rondani, 1845. Many recent papers follow this interpretation of *Eristalinus*, but *Eristalodes* and *Lathyrophthalmus* are also treated as genera by some authors (eg.: Dirickx, 1998), or as belonging to the genus *Eristalis sensu lato* (eg.: Verlinden, 1994). Thus, the classification of the eristalines is in a state of flux.

Five *Eristalinus* species are listed for Europe in the Catalogue of Palaearctic Diptera (Peck, 1988): *E. aeneus* (Scopoli, 1763), *E. megacephalus* (Rossi, 1794), *E. quinquelineatus* (Fabricius, 1781), *E. sepulchralis* (Linnaeus, 1758) and *E. taeniops* (Wiedemann, 1818). Dirickx (1994) showed that European records of *E. quinquelineatus* were erroneous and referred to *E. megacephalus*, thus reducing the European fauna to four species. Two of them do not reach further north than the Mediterranean basin (*E. megacephalus* and *E. taeniops*), but the other two (*E. aeneus* and *E. sepulchralis*) are more widely distributed species (Speight, 2001).

Although species of *Eristalinus* are common and often numerous, their preimaginal morphology is poorly known. Only the immature stages and life history of *E. aeneus* and *E. sepulchralis* are described (Metcalf, 1913; Klein-Krautheim, 1936; Dixon, 1960; Hartley, 1961). The rearing records indicate that the larva of *E. aeneus* is saprophagous, occurring in coastal rock pools containing

* Corresponding author. E-mail: celeste.perez@ua.es

TABLE 1. Tribe Eristalini: species included in the molecular study. +[a] = partial sequence.

Species	Locality	COI	28S
Eristalinus aeneus (Scopoli)	Spain, Alicante (salt water)	+	–
E. aeneus (Scopoli)	Greece, Lesbos	+	+
E. dubiosus Curran	Kenya, Kakamega forest	+[a]	+
E. megacephalus (Rossi)	Spain, Alicante	+	+
E. sepulchralis (Linnaeus)	Estonia, Saaremaa	+	+
E. taeniops (Wiedemann)	Greece, Lesbos, Thermi	+	+
Eristalis tenax (Linnaeus)	Europe: multiple localities	+	+
Helophilus trivittatus (Fabricius)	Greece, Lesbos, Thermi	+	+
Myathropa florea Linnaeus	Greece, Lesbos, Sikaminia	+	+
Phytomia incisa Wiedemann	Kenya, Kakamega forest	+	+
Eumerus etnensis Van der Goot	Spain, Alicante	+	+

large amounts of decaying seaweed (Hartley, 1961). *E. sepulchralis* occurs in accumulations of decaying vegetation in ponds, pools and marshes and also in wet manure (Hartley, 1961). Information on the habits, biology and ecology of *E. taeniops and E. megacephalus* is virtually lacking. Less than 15% of the larvae of the tribe Eristalini are known, and as is well known, the characters of the immature stages have proven important and informative both at the specific and higher taxonomic levels (Rotheray & Gilbert, 1999; Marcos-García & Pérez-Bañón, 2001).

The aim of this study is to describe the preimaginal morphology of the European species of *Eristalinus*, which are undescribed (*E. taeniops* and *E. megacephalus*) and re-describe those of the two other species (*E. aeneus* and *E. sepulchralis*), including for the first time a description of the thoracic respiratory process of *E. aeneus*. We also describe and compare the male genitalia of *E. taeniops* and *E. megacephalus* for the first time.

As different taxonomic criteria were used for evaluating the adult morphological differences, it is difficult to establish to which group (including rank) the species "belong". This prompted us to employ novel pre-imaginal and molecular evidence to increase the understanding of the classification and the taxonomic status of the European species. The DNA sequence characters of various genes are increasingly used for resolving species-level and higher taxonomic level problems. We employed a partial sequence of the mitochondrial gene cytochrome *c* oxidase subunit I (COI), and a partial sequence of the nuclear ribosomal gene 28S rRNA, which are frequently used for determining insect molecular phylogenies.

MATERIAL AND METHODS

Adult morphological studies

We studied numerous specimens of males and females of all the European species of the genus *Eristalinus* in the entomological collections of Universidad de Alicante, Alicante, Spain (CEUA) and Finnish Museum of Natural History, Helsinki, Finland (FMNH). We have also studied part of the material used by Kanervo in 1938, which is present in the Finnish Museum of Natural History. The specimens were mainly from Europe, but material from Afrotropical region was also studied.

Male genitalia. Illustrations were made of preserved material using a binocular microscope with an eyepiece micrometer and FSA 25 PE drawing tube. The male genitalia was drawn in glycerine after clearing in warm potassium hydroxide (KOH) for 3–4 minutes and washing in distilled water. The terminology used for the various parts of the genitalia follows Hippa et al. (2001).

Larval morphological studies

We used numerous larvae and pupae of *Eristalinus* species from the entomological collection of Universidad de Alicante, Spain (CEUA) and the National Museum of Scotland, Edinburgh, U.K. (NMS). Field collected larvae were placed in plastic cages containing water from where they were collected. Rearing took place in a growth chamber at 16–22°C, 80 ± 5% r h and a constant photo-regime of 15L : 9D. Pupae were isolated in individual Petri dishes and inspected daily until the emergence of the adults. The larvae were collected mainly at localities in the West and East Mediterranean (Alicante province, Spain and Lesbos Island, Greece). Larvae selected for preservation were in the third stage. For permanent preservation, larvae were killed by immersion in cold water and heated slowly for about four minutes to extend them. Afterwards, they were preserved in 70% alcohol.

Descriptions are based on preserved specimens with larval characters checked against living specimens in order to minimise errors due to preservation. Illustrations and dimensions (mean ± standard error) were made on preserved material using a binocular microscope with an eyepiece micrometer and FSA 25 PE drawing tube. The photographs were taken with a scanning electron microscope (SEM) operated at 20 kV.

Terminology used for descriptions of the larvae follows Hartley (1961) and Rotheray (1993). The positions of the sensilla were numbered sequentially from the dorsal to the ventral surface for each segment (Rotheray, 1991).

Molecular studies

DNA was extracted from dry, pinned specimens or from specimens preserved in 70–95% alcohol (Table 1). Male terminalia are conserved for the morphological studies. DNA was extracted from parts of single individual (usually its legs) using the NucleoSpin Tissue DNA Extraction kit (Machery-Nagel) and resuspended in 50 µl MilliQ water.

PCRs were carried out in 25 µl reactions containing 1 µl DNA extract, 1 µl of primer (concentration of 10 pmol/ml), 1 U of Amplitaq DNA polymerase, 2 µl 25 mM $MgCl_2$, 2.5 µl of 10X Buffer II (Applied Biosystems) and 200mM dNTP (GeneAmp). PCR conditions were initial denaturing at 95°C for 2 min, 29 cycles of 30 s denaturing at 94°C, 30 s annealing at 49°C, 2 min extension at 72°C and a final extension 8 min at 72°. PCR products were purified using GFX PCR Purification Kit (Amersham Biotech) and then sequenced (using the PCR primers) in both directions using the Big Dye Terminator Cycle Sequencing kit (Applied Biosystems) at one-fourth of the recommended volumes on an ABI PRISM 377 automated DNA

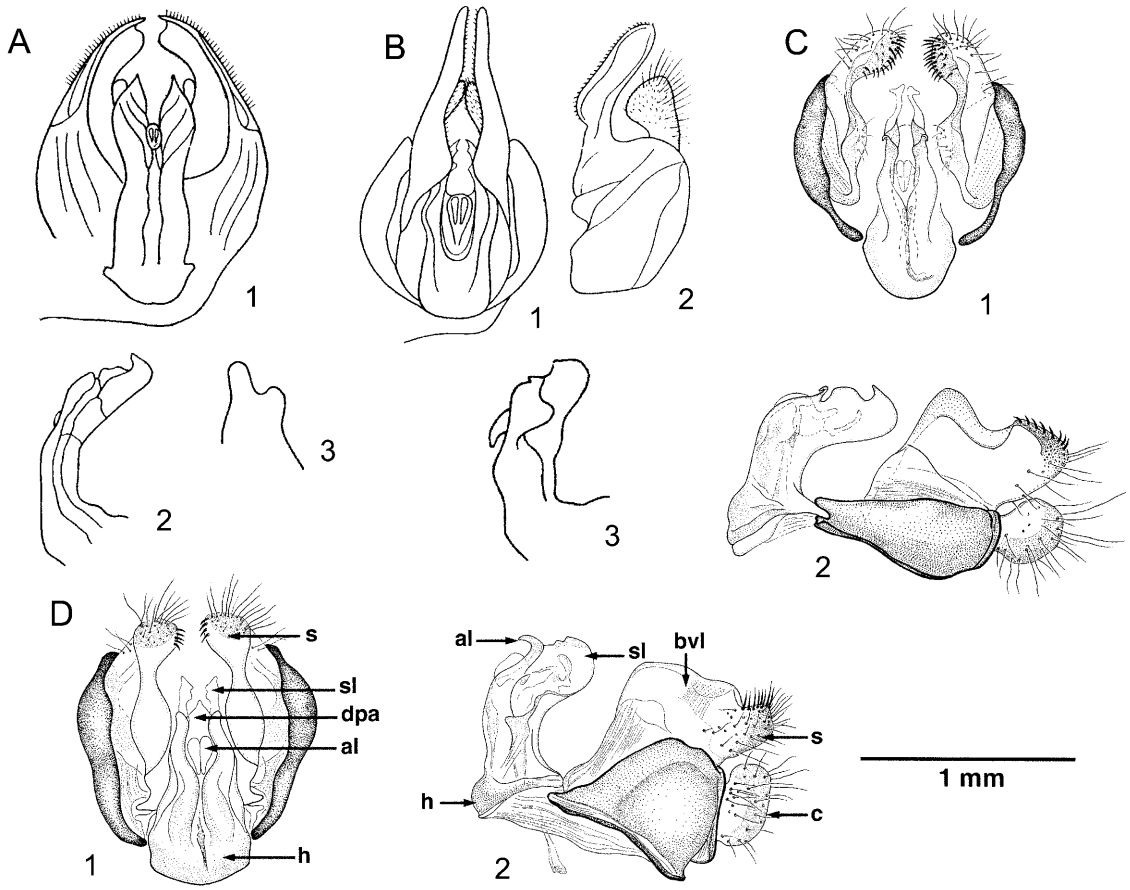

Fig 1. Male genitalia of *Eristalinus*. A – *E. aeneus,* ventral view (1), hypandrium, lateral view (2), apical part of surstylus, ventral view (3); B – *E. sepulchralis,* ventral view (1), surstylus, lateral view (2), hypandrium, lateral view (3); C – *E. taeniops*, ventral (1) and lateral view (2); D – *E. megacephalus*, ventral (1) and lateral view (2). A and B modified from Kanervo (1938). al – aedeagal lobe; bvl – basoventral lobe of surstylus; c – cercus; dpa – dorsal part of the aedeagal lobe; h – hypandrium; s – surstylus; sl – superior lobe of hypandrium.

sequencer. The primers used for amplifying and sequencing COI and the D2-3 region of 28S are listed in Table 2. The sequences were assembled and inspected using Sequence Navigator™(version 1.01).

For this paper we sequenced mainly European species of *Eristalinus* but some taxa from the Afrotropical region were also sequenced.

In the COI gene there was no ambiguity caused by gaps, the D2-3 region of the 28S gene was manually aligned. Parsimony analysis was performed on the combined data matrix using the program Nona vs. 2.0 (Goloboff, 1993), (with commands hold*; hold/20; mult*200) and branch support (Bremer support) was calculated using the same program. *Eumerus etnensis* van der Goot, 1964 (Eumerini) was used as outgroup.

RESULTS

DESCRIPTION OF THE MALE GENITALIA

We include here a full description of the male genitalia of *E. megacephalus* as Shatalkin (1971) provides only a partial drawing. As some pieces of the hypandrium of *E. taeniops* are not properly detailed in the figures in Kanervo's paper (1938), we also include a description of the genitalia of this species.

Eristalinus taeniops (Wiedemann, 1818)

Ventral view (Fig. 1C1)

The inner sides of the surstylus are noticeably sinuous. Superior lobes clearly with sinuous inner and upper sides and with acute and peak shaped extremities. The dorsal part of the aedeagus is regularly convex.

Lateral view (Fig. 1C2)

The surstylus of *Eristalinus taeniops* in lateral view has a very prominent and well-developed basoventral lobe. Hypandrium of *Eristalinus taeniops* evenly broad, with the superior lobe rounded apically and with one conspicuous subventral projection. Aedeagal lobe ventrally incurved with a small ventral hook.

Material examined. 5 males, Thermi (Lesbos) Greece coll. 8.IV.2001, leg.: C. Pérez-Bañón & S. Rojo (CEUA); 2 males, San Vicente del Raspeig (Alicante) Spain, coll. VI.1995, leg.: J. Ordóñez (CEUA); 2 males, El Torno (Cáceres) Spain, coll. 25.VIII.1980, leg.: M. A. Marcos-García (CEUA). 1 male, Luxor Egypt (44628. II), *Eristalis taeniops* Wiedem det.: Becker (FMNH); 1 male, Ishurum ? 1.4.36. F. Brandt, C.1.4. (FMNH); 2 male, 505, 507, Gr. Canaria, Tafira, R. Frey (FMNH).

Eristalinus megacephalus (Rossi, 1794)

Ventral view (Fig. 1D1)

TABLE 2. Primers used for PCR and sequencing.

Primer	Sequence	Source
HCO-N-2198	5'-GGTCAACAAATCATAAAGATATATTGG-3'	Folmer et al. 1994
C1-S-1718	5'-GGAGGATTTGGAATTGATTAGTTCC-3'	Simon et al. 1994
C1-J-2183	5'-CAACATTTATTTTGATTTTTTGG-3'	Simon et al. 1994
TL2-N-3014	5'-TCCAATGCACTAATCTGCCATATTA-3'	Simon et al. 1994

The inner sides of the surstylus sinuous. The upper side of the superior lobe of the aedeagus is straight and the inner margin indentate. Dorsal part of the aedeagal lobe (dpa) acute apicoventrally.

Lateral view (Fig. 1D2)

Basoventral lobe of surstylus (bvl) regularly prominent. Apical extreme of the surstylus (s) rounded and with black hairs and setulae reclined to the inner side. Cercus (c) sub-rectangular, two times wider than high. Hypandrium (h) curved dorsally, with the superior lobe (sl) evenly broad, not constricted basally, rounded apically and with three small teeth ventrally. Aedeagal lobe (al) ventrally incurved and hook shaped.

Diagnosis. The genitalia of this species presents the diagnostic character, described by Kanervo (1938), as typical of the species belonging to genus *Lathyrophthalmus* (*sensu* Kanervo): "Stylus with a well developed lobe in the inner side". This pattern resembles closely that of *Eristalinus taeniops*, but differ in the shape of the surstylus, the distal part of the superior lobe, the aedeagal lobe and the dorsal part of the aedeagus.

Material examined. 1male, Orihuela (Alicante), Spain, coll. 29.V.2001, leg.: M. Louis (CEUA); 1 male, Doñana (Huelva) Spain, coll. 17.X.1983 leg.: C. Herrera (CEUA); 1male, La Calzada de Béjar (Salamanca) Spain, coll. 28.IX.1977, leg.: S. Fernández-Gayubo (CEUA); 3 males, San Vicente Rib. Juliao, Ins. Cabo Verde coll. 26.11-2.12.1953 Lindberg, *Eristalinus megacephalus* Rossi, det.: C. Claussen 1985 (FMNH).

DESCRIPTION OF THE IMMATURE STAGES

We provide a full description of the larva and puparium of *E. taeniops*, and of the puparium of *E. megacephalus*. For *E. sepulchralis* and *E. aeneus*, we describe the morphology of the parts previously undescribed, and the morphology of the parts that differ from the previous species.

***Eristalinus taeniops* (Wiedemann, 1818)**

Third larval stage

Length not including prp 1.8 ± 0.04 cm, maximum width 4.2 ± 0.08 mm (n = 10). Overall appearance: A long-tailed larva with internal mouth-hooks and a retractile anterior spiracle. Sub-cylindrical in cross-section with a flattened ventral surface, truncate anteriorly and tapering posteriorly. Dorsal body surface coated in fine pubescence directed posteriorly, pubescence becoming longer around the lateral sensilla (0.15 mm to 0.30 mm). Setae on ventral surface are short except on the anal segment. Ventro-lateral margin of abdominal segments bearing one line of long setae (0.37 mm). Sixth abdominal segment bearing a transverse row of spicules immediately in front of the last pair of prolegs. Prolegs bearing crochets in 2 main rows.

Head. Mandibles and mandibular lobes internal, mandibles supporting the expanded mandibular lobes [mouthparts adapted for filter-feeding (Roberts, 1970)]. Basal section of the papilla supporting antenno-maxillary organs divided to the base. Antenno-maxillary organs well developed.

Thorax. Dorsal lip broad, lacking a medial groove and covered with a conspicuous tuft of long setae. Lateral lips rounded and well-developed (in profile projecting forward from the anterior part of the prothorax) coated in short and fine setae at base and longer setae at tip. Dorsal surface of the prothorax with longitudinal grooves. Anterior fold with a broad band of backwardly directed spicules, which are slightly hooked, and sclerotized on the distal half, and become progressively shorter posteriorly. Dorsal surface of the prothorax with a pair of spiracles about three times higher than broad, light brown in colour, sclerotized, with pointed slightly recurved tips, and retractile into inverted integumental pockets. Spiracular openings are situated on a clear area of the ventral surface, extending along the distal three fourths of spiracle length. The clear area is about two times longer than broad, with a fold on the apical third (Fig. 2A). The unsclerotized area is almost entirely obliterated by facets, only a small part on the apical end without facets. The facets are arranged in a band of four or five rows up to the fold (Fig. 2A). Lateral margin of mesothorax with two patches of sclerotised spicules arranged as follows: a group with 10–15 spicules immediately anterior to 4th sensilla of mesothorax and another group with 25 spicules located in front of the 5th sensilla of mesothorax. Mesothorax bearing well developed prolegs with about 60 crochets arranged in multiple rows.

Abdomen. Primordia of pupal spiracles obvious on the dorsal surface of first abdominal segment. Six pairs of ventral prolegs on segments 1–6. Prolegs well developed, in ventro-lateral view, appear as small cones that are broader at the apex than the base, with crochets in 2 rows, backed by several rows of stout spinules. About 6–7 primary crochets long and slender, with the distal third sclerotized, these crochets are bigger than posterior ones. Last pair of prolegs with most of the large primary crochets facing towards the lateral margins of the body. A short double transverse row of slender, anteriorly directed spicules in front of the last pair of prolegs, 4–5 of the posterior row being much larger than the others. On segment 7 sensilla 4 is aligned horizontally with sensilla 5 and 6. Ventro-lateral margin of the abdominal segments 1–7 with one line of long and aggregated setae at level of sen-

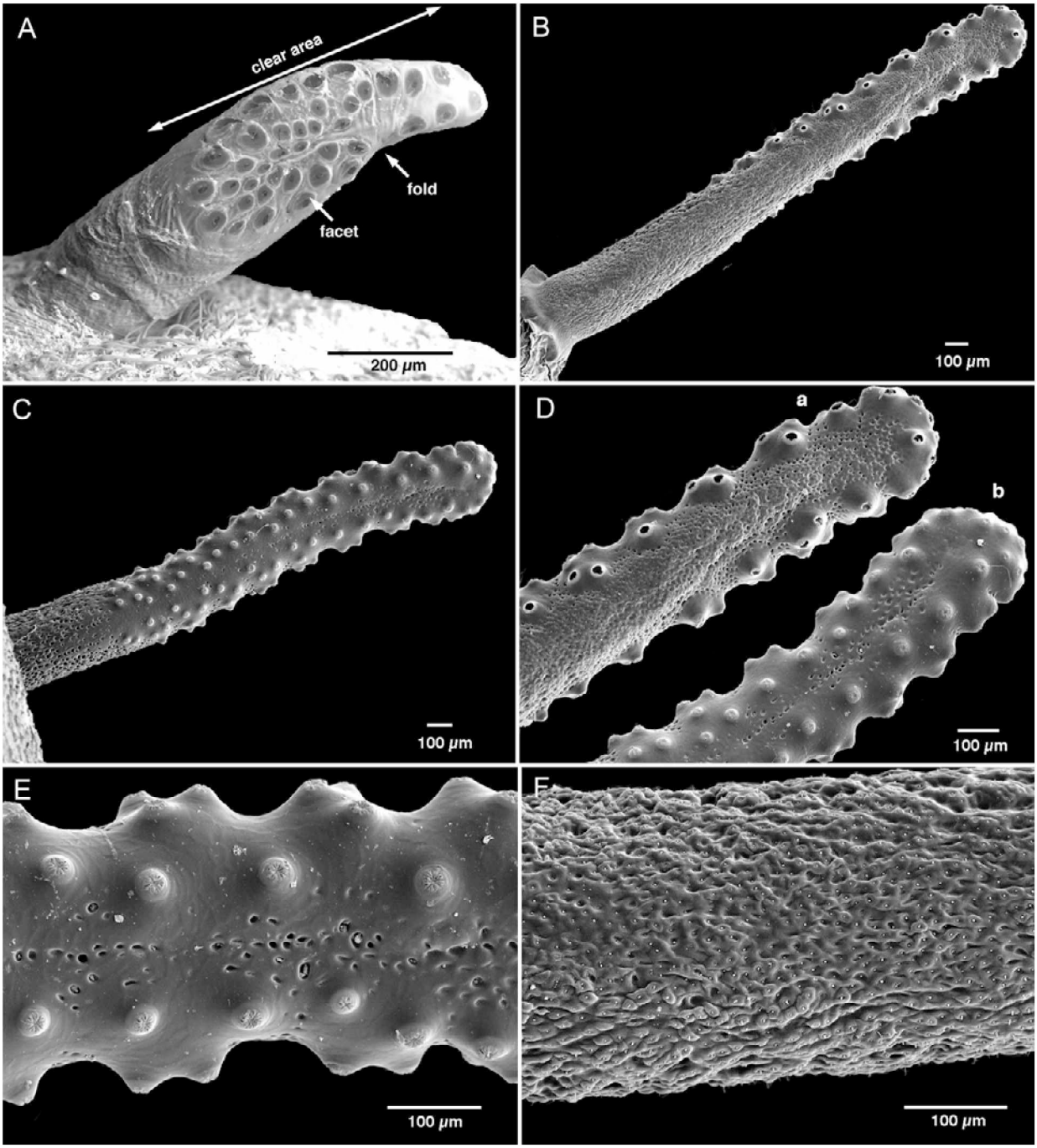

Fig. 2. *Eristalinus taeniops*. A – anterior larval spiracle, dorso-lateral view; B-F – pupal spiracle. B – ventral view; C – dorsal view; D – distal view of ventral (a) and dorsal (b) surfaces; E – spiracular openings; F – ornamentation on basal part.

silla 7–8. Anal segment extended as in long tailed larvae, with three pairs of weakly developed lappets. Posterior respiratory process (prp): shiny, sclerotized and brown in colour.

Chaetotaxy

Prothorax with 12 pairs of sensilla; mesothorax and metathorax with 9 pairs; abdominal segments 1–7 with 11 pairs; anal segment with 3 pairs of sensilla (sensilla 9, 10 and 11) and three pairs of lappets (similar to *E. sepulchralis*, illustrated by Hartley, 1961).

Puparium

Sub-cylindrical in cross-section. Anterior end truncate, tapered posteriorly and flattened ventrally. Dark brown in colour. Pupal spiracles projecting from middle of upper part of operculum, separated by a distance of more than half the length of one spiracle. These processes are sub-cylindrical structures about 2.25 mm in length (length width ratio of spiracle 9:1) (Fig. 2B, C), facing towards the lateral margins of the puparium and with the distal four fifths progressively flattened apically. Dark brown in colour. About 80% of the dorsal and lateral surfaces with irregularly spaced and oval-shaped tubercles each bearing

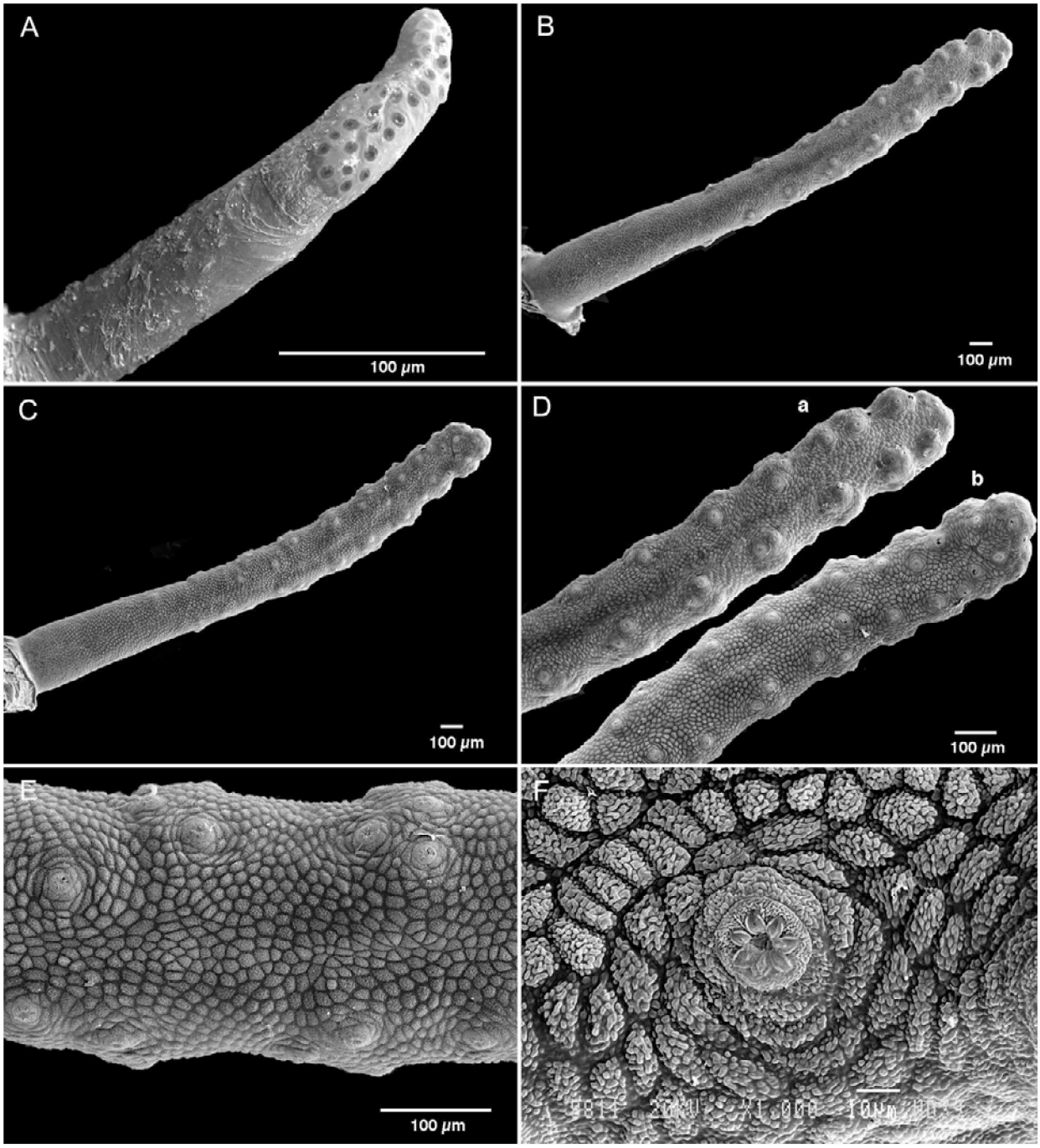

Fig. 3. *Eristalinus aeneus*. A – anterior larval spiracle, dorso-lateral view; B-F – pupal spiracle. B – ventral view; C – dorsal view; D – distal view of ventral (a) and dorsal (b) surfaces; E, F – spiracular openings.

6–10 oval spiracular openings (Fig. 2C, E). The tubercles are arranged in 7–8 vague bands laterally, which are slightly swollen. Dorsal surface with a narrow tapering gap, which disappears at the base (Fig. 2C, D). Entire surface of the pupal spiracles, except the space between tubercles, finely reticulated with clearly concave "cells" bearing short apical setae at least on the basal half (Fig. 2F). Surface between the tubercles smooth (Fig. 2E).

Material examined. 15 larvae, San Vicente del Raspeig (Alicante), Spain, coll. VI.1995 leg.: J. Ordóñez (CEUA); 15 larvae, Thermi (Lesbos), Greece, coll. 8.IV.2001 leg.: C. Pérez-Bañón & Rojo, S. (CEUA); 20 puparia (15 males + 5 females) Thermi (Lesbos), Greece, coll. 8. IV.2001 leg.: C. Pérez-Bañón & Rojo, S. (CEUA); 6 puparia (2 males + 4 females) San Vicente del Raspeig (Alicante), Spain, coll. VI.1995 leg.: J. Ordóñez (CEUA).

Eristalinus aeneus (Scopoli, 1763)

Third larval stage

Anterior larval spiracles. Spiracles about five times longer than broad (Fig. 3A). Light reddish brown in colour. Spiracular openings on a clear area on the ventral surface, which extends to the distal third of the spiracle (Fig. 3A). Clear area is about three times longer than broad, with a fold above the middle of its length. Facet

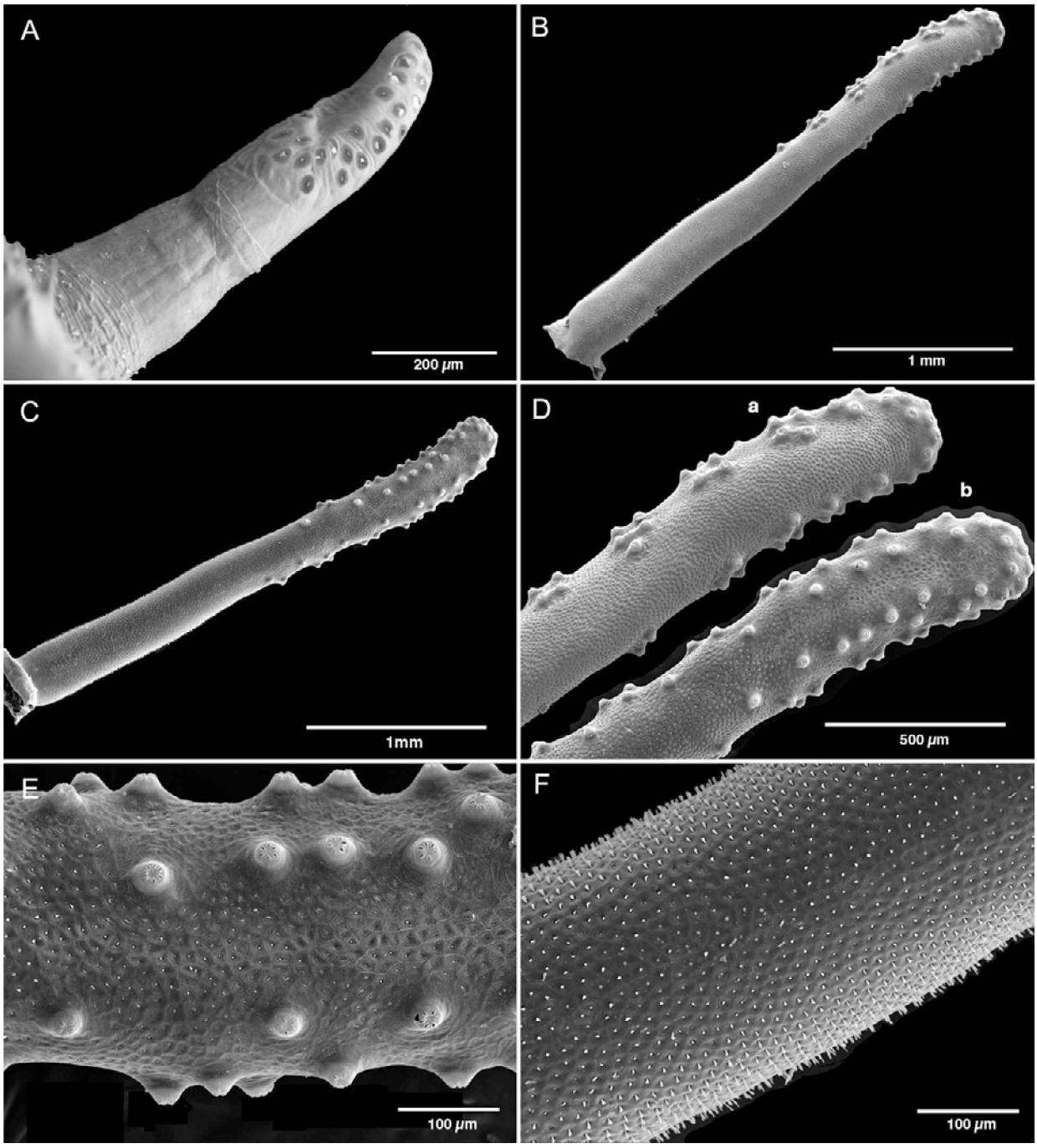

Fig. 4. *Eristalinus sepulchralis*. A – anterior larval spiracle, dorso-lateral view; B-F – pupal spiracle. B – ventral view; C – dorsal view; D – distal view of ventral (a) and dorsal (b) surfaces; E – spiracular openings; F – ornamentation on basal part.

band arranged in two or three rows below the fold (Fig. 3A).

Puparium

Sub-cylindrical in cross-section. Anterior end truncate, tapered posteriorly and flattened ventrally. Dark brown in colour. Pupal spiracles projecting from middle of upper part of operculum, separated by a distance close to half the length of one spiracle. These processes are sub-cylindrical structures about 2.25 mm in length (length breadth ratio of spiracle 9:1) and face towards the lateral margins of the puparium (Fig. 3B, C). Light brown in colour. About 65 % of the dorsal and lateral surfaces with irregularly-spaced and oval-shaped tubercles, each bearing 6–10 oval spiracular openings (Fig. 3C, F). The tubercles are arranged in 7-8 vague bands laterally, which are slightly swollen (Fig. 3C, D). The distance between the bands increases basally. Dorsal surface bearing a broad gap the entire spiracular length (Fig. 3B, C). Entire surface of the pupal spiracles, including the space between tubercles, finely reticulated with convex "cells" without apical setae (Fig. 3E, F).

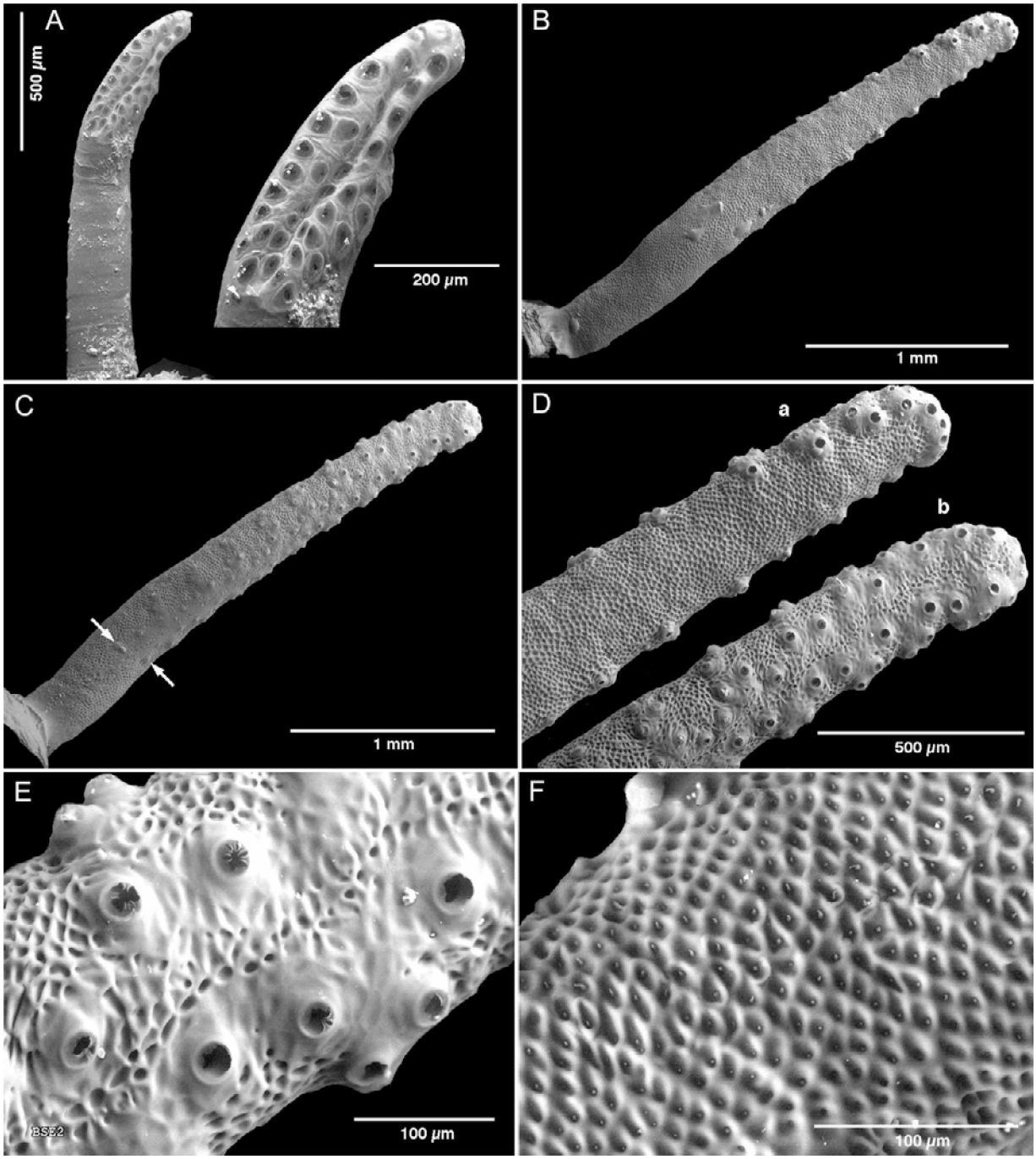

Fig. 5. *Eristalinus megacephalus*. A – anterior larval spiracle, dorso-lateral view; B-F – pupal spiracle. B – ventral view; C – dorsal view; D – distal view of ventral (a) and dorsal (b) surfaces; E – spiracular openings; F – ornamentation on ventral surface.

Material examined. 10 larvae, Orihuela (Alicante), Spain, coll. 29.V.2001 leg.: M. Louis (CEUA); 10 larvae, Vatousa (Lesbos), Greece, coll. 12.V.2001 leg.: C. Pérez-Bañón & Rojo, S. (CEUA); 8 puparia (4 males + 4 females) Vatousa (Lesbos), Greece coll. 12.V.2001 leg.: C. Pérez-Bañón & Rojo, S. (CEUA); 8 puparia (4 males + 4 females) Orihuela (Alicante), Spain, coll. 29.V.2001 leg.: M. Louis (CEUA).

Eristalinus sepulchralis (Linnaeus, 1758)

Third larval stage

Anterior larval spiracles. Spiracles about three times taller than broad (Fig. 4A). Light reddish brown in colour. Spiracular openings on a clear area on the ventral surface, which extends to the distal two fifths of the spiracle (Fig. 4A). Clear area is about two times longer than broad, with the fold below the middle of its length (Fig. 4A). Facet band arranged in two rows.

Puparium

Sub-cylindrical in cross-section. Anterior end truncate, tapered posteriorly and flattened ventrally. Light to dark brown in colour. Pupal spiracles projecting from middle of upper part of operculum, separated by a distance of about a third of the spiracle length. These processes are

TABLE 3. Pair-wise uncorrected sequence distances calculated for *Eristalinus* species.

Species		Percentage
E. megacephalus vs	*E. taeniops*	5.8%
	E. aeneus	7.8%
	E. sepulchralis	8.4%
E. taeniops vs	*E. aeneus*	7.8%
	E. sepulchralis	8.3%
E. aeneus vs	*E. sepulchralis*	5.3%

sub-cylindrical structures of about 2.8 mm in length (length width ratio of spiracle 9:1) face towards the lateral margins of the puparium and are slightly flattened apically (Fig. 4B, C). Dark brown in colour. About 50% of the dorsal and lateral surface with irregulary-spaced and oval-shaped tubercles each bearing 6–10 oval spiracular openings (Fig. 4C, E). The tubercles are arranged in 5–7 vague bands laterally, which are not swollen (Fig. 4C, D). Distance between bands increases basally. Dorsal surface bearing a broad gap the entire spiracular length (Fig. 4C). Entire surface of the pupal spiracles, including the space between tubercles, finely reticulated with slightly concave "cells" bearing apical setae at least on the basal half (Fig. 4E, F).

Material examined. 5 larvae, 5 puparia (NMS).

Eristalinus megacephalus (Rossi, 1794)

Third larval stage

Anterior larval spiracles. Spiracles about five times longer than broad (Fig. 5A). Light reddish brown in colour. Spiracular openings on a clear area on the ventral surface, which extends to the distal third of the spiracle. Clear area is about three times longer than broad, with the fold above the middle of its length (Fig. 5A). Unsclerotized area almost entirely obliterated by facets. Facet bands arranged in four or five rows below the fold (Fig. 5A).

Puparium

Sub-cylindrical in cross-section. Anterior end truncate, tapered posteriorly and flattened ventrally. Light to dark brown in colour. Pupal spiracles projecting from middle of upper part of operculum, approximately separated by distance equal to a third of the spiracle length. These processes are sub-cylindrical structures about 2.6 mm in length (length breadth ratio of spiracle 9:1), face towards the lateral margins of the puparium and are slightly flattened apically (Fig. 5B, C). Clearly brown in colour. About 80% of the dorsal and lateral surface with irregulary-spaced and oval-shaped tubercles each bearing 4–7 oval spiracular openings (Fig. 5C, E). The tubercles are arranged in 7–9 vague bands laterally, which are not swollen (Fig. 5C, D). Entire surface of the pupal spiracles, including the space between tubercles, finely reticulated with clearly concave "cells" bearing short apical setae at least on the basal half (Fig. 5F). Only the surface around the tubercles is smooth (Fig. 5E).

Material examined. 1 puparium (1male) Orihuela (Alicante), Spain, coll. 29.V.2001 leg.: M. Louis (CEUA).

KEY TO EUROPEAN *ERISTALINUS* PUPARIA

1 Pupal spiracles with less than the distal three quarters of the dorsal and lateral surface covered by tubercle bands, indistinct dorsally, distinct laterally (Fig. 3C, 4C) 2
– Pupal spiracles with more than the distal three quarters of the dorsal and lateral surface covered by tubercle bands, indistinct dorsally, distinct laterally (Fig. 2C, 5C) 3
2 Entire surface of the pupal spiracles, including the space between tubercles, finely reticulated with concave "cells" bearing apical setae at least on the basal half (Fig. 4E, F) .. .. *E. sepulchralis*
– Entire surface of the pupal spiracles, including the space between tubercles, finely reticulated with convex "cells" without apical setae (Fig. 3E, F) ..... *E. aeneus*
3 Pupal spiracles with tubercles arranged in 7–8 vague bands laterally, which are slightly swollen (Fig. 2B, C, D) .... *E. taeniops*
– Pupal spiracles with tubercles arranged in 7–9 vague bands laterally, which are not swollen (Fig. 5B, C, D) .... *E. megacephalus*

Life history

The larvae of *E. aeneus*, *E. megacephalus* and *E. taeniops* were found together with other eristaline species in streams rich in organic matter. In the West Mediterranean localities the origin of this organic matter was sewage from farms (pig farming) or sewage from olive oil factories. The larvae of *E. taeniops* were also found in pools containing decaying carcasses of animals or exclusively with decaying plant matter (rain water plus pine pollen and needles) and those of *E. aeneus* in pools of hypersaline water containing seaweed. The eggs were laid in clusters of up to a hundred at the edges of streams under and between the stones situated near the water. Pupation took place in vegetation and stones slightly above the water level. In the case of *E. taeniops* we observed a strange gregarious behaviour just before pupation, resulting in a mass of pupae with the posterior respiratory processes interlaced in the inner part.

MOLECULAR STUDIES

Altogether 1128 nucleotide characters were obtained from the COI spanning nucleotide positions 1777 to 2903 (numbering is based on *Drosophila yakuba* sequence; Clary & Wolstenholme 1985). The aligned data matrix of the D2-3 region of the 28S rRNA gene comprised 642 sites.

The uncorrected pairwise sequence divergence in the COI gene between *E. sepulchralis* and *E. aeneus* was 5.3%, and 5.8% in the case of *E. taeniops vs E. megacephalus*. On the other hand, the variation between *E. taeniops* or *E. megacephalus* and *E. aeneus* or *E. sepulchralis* varied from 7.8 to 8.4% (Table 3). The sequences from the *E. aeneus* specimens were identical.

Parsimony analysis of the combined data matrix resulted in two most parsimonious trees of length 740 steps, CI = 0.68, RI= 0.40. The strict consensus tree of these is shown in Fig. 6. The incomplete sequence data for *E. dubiosus* probably produced the unresolved apical polytomy.

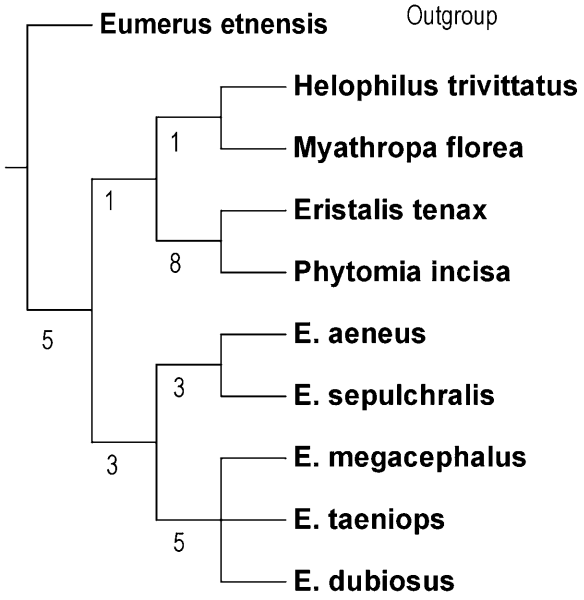

Fig. 6. Strict consensus of the two most parsimonious trees resulting from combined parsimony analysis of DNA sequence data of mitochondrial COI and nuclear ribosomal 28 rRNA genes, length 740 steps, CI = 0.68, RI = 0.40. Branch support values indicated below nodes.

DISCUSSION

According to current taxonomic information on the genus *Eristalinus*, the Palaearctic species are easily identified because the males of the subgenera *Eristalodes* and *Lathyrophthalmus* are holoptic, while the males of the subgenus *Eristalinus* (*sensu stricto*) are dichoptic. Moreover, the representatives of *Eristalodes* are separated from *Lathyrophthalmus* by the presence of bands (fasciae), instead of dark spots (punctate) on the eyes.

Kanervo (1938) found interspecific variability in the structure of the male genitalia in some Palaearctic species of *Eristalis* (*sensu lato*). He figured this variation in the male genitalia and used it to arrange the species of *Eristalinus*, *Eristalodes* and *Lathyrophthalmus* in three groups. This arrangement did not affect the *Eristalinus*-group because the male genitalia of *Eristalinus sepulchralis* is clearly different from that of the other groups. However, he discovered that the structure of the male genitalia of *Eristalodes taeniops* (with fasciate eyes) is similar to that of *Lathyrophthalmus quinquestriatus* (Fabricius, 1794) (with punctate eyes). Moreover the structure of the genitalia of *Lathyrophthalmus aeneus* (punctate eyes) is different from that of the above species. He used the name of *Lathyrophthalmus* Mik (syn. *Eristalodes* Mik, *sensu* Kanervo) for the second species-group and created a new name, *Metalloeristalis* Kanervo, 1938, exclusively for the *aeneus*-group. Kanervo did not know the structure of the male genitalia of the European *E. megacephalus* (= *quinquelineatus* of Kanervo) but suggested that this species could belong to the *taeniops/quinquestriatus*-group. These taxonomic arrangements were largely ignored, except for the monophyly of *Eristalinus* + *Lathyrophthalmus* + *Metalloeristalis* (group VI of Kanervo).

Based on larval morphology, the four European species of *Eristalinus* (*sensu lato*) can be distinguished from other syrphid larvae by the presence of a short double row of crochets immediately in front of the sixth abdominal prolegs. Hartley (1961) used this character to separate the larvae of *E. sepulchralis* and *E. aeneus* from the other species of Eristalini. Later Rotheray (1993) considered this feature a diagnostic character of the genus *Eristalinus*, but he did not study any other species. The fact that the larvae of the two species described in this paper also possess this character supports the monophyly of this group. This character is not present in other eristalines although a few scattered spicules may be present between the last pair of prolegs in some genera (Rotheray & Gilbert, 1999).

The pupae of these four species are easily distinguished from each other using the characters of the anterior larval spiracles (Table 4) and the puparia using the ornamentation on the thoracic respiratory processes (key to *Eristalinus* puparia).

According to the ornamentation on the thoracic respiratory processes and the number and arrangement of facets in the anterior larval spiracles, *E. megacephalus* (Fig. 5A-F) seems to be more closely related to *E. taeniops*

TABLE 4. Comparison of the main morphological characters of third-instar larvae of European species of *Eristalinus*.

Characters	*E. taeniops*	*E. aeneus*	*E. sepulchralis*	*E. megacephalus*
Anterior larval spiracles				
Size	three times taller than broad (Fig. 1A)	three times taller than broad (Fig. 1A)	three times taller than broad (Fig. 1A)	five times taller than broad (Fig. 4A)
Size of the clear area on the spiracular ventral surface	which extends to the distal three quarters of spiracle length (Fig. 1A)	which extends to the distal third of the spiracle (Fig. 2A)	which extends to the distal two fifths of the spiracle (Fig. 3A)	which extends to the distal third of the spiracle (Fig. 4A)
Facet band	arranged in four or five rows below the fold (Fig. 1A)	arranged in two or three rows below the fold (Fig. 2A)	arranged in two rows (Fig. 3A)	arranged in four or five rows below the fold (Fig. 4A)
Abdomen				
Two transverse rows of spicules immediately in front of the last pair of prolegs	with seven to nine large crochets in the posterior row	with seven to nine large crochets in the posterior row	with only five large crochets in the posterior row	with seven to nine large crochets in the posterior row

(Fig. 2A-F) than to *E. aeneus* (Fig. 3A-F). This is not supported by the size of the anterior larval spiracles, a character that is very variable in the species of other eristaline genera (Pérez-Bañón et al., 2003).

The results of our studies on the morphology of the male genitalia (Fig. 1) and the molecular data also corroborate the close relationship of *E. megacephalus* and *E. taeniops*. These results do not support the traditional classification of adults based on the patterning on the eyes (fasciate *vs* punctate). This could be related to the fact that in *E. aeneus*, vision is not influenced by the patterning on the eyes, as the cornea transmits all wavelengths of light involved in vision equally well (Knüttel & Lunau, 1997). We therefore hypothesize that the patterning on the eyes is not a taxonomically important character in *Eristalinus* (*sensu lato*) and the classification of the species of this genus using this character should be abandoned.

This study of the male genitalia and the morphology of the immature stages of the European species, support the three groups of Kanervo (*E. sepulchralis*, *L. aeneus* and *E. taeniops* + *L. megacephalus*). Moreover the molecular studies indicate that *E. sepulchralis* and *L. aeneus* are closer related than they are to either *E. taeniops*, and *L. megacephalus* (Fig. 6).

Accordingly we propose that the characters of the male genitalia used by Kanervo (1938) for designating the *Eristalinus-Eristalodes-Lathyrophthalmus* European species complex are important taxonomic features and can be used to classify these species. Unfortunately this author did not study the relevant type material and so Kanervo's names are not correct according to the Zoological Code (Art. 61.1). The valid names must be: *Eristalinus* Rondani, 1845 species type *E. sepulchralis* (Linnaeus, 1758); *Eristalodes* Mik, 1897 (Syn. *Lathyrophthalmus* sensu Kanervo, 1938) species type *E. taeniops* (Wiedemann, 1818) (syn *taeniopus*, lapsus) and *Lathyrophthalmus* Mik, 1897 (Syn. *Metalloeristalis* Kanervo, 1938) species type *L. aeneus* (Scopoli, 1763).

The results of the molecular studies support two clades, and these could be two genera/subgenera: *Eristalodes* (including *E. taeniops* species type and *E. megacephalus*) and *Eristalinus* (including *E. sepulchralis* species type and *E. aeneus*) (Fig. 6). According to the genitalia figured in Kanervo's (1938) paper, the genus *Eristalodes* also includes some West Palaearctic species such as *E. quinquestriatus* (Fabricius, 1794) and *E. tarsalis* (Macquart, 1855). However, further studies of adult morphology, male genitalia, pre-imaginal morphology and molecular data are needed before reviewing the taxonomic status of other species, which may reveal new groups.

ACKNOWLEDGEMENTS. We are greatly indebted to the following persons for the loan of, or for providing specimens, used in the present study: Graham Rotheray (U.K), Jeroen van Steenis (Sweden) and Theodora Petanidou (Greece). Illustrations were kindly produced by Alma B. Gámez. Financial support was provided by the Spanish Ministry of Science and Technology (AGL 2000-0342-P4-02 and BOS 2000-0148) and Universidad de Alicante, Spain (GR02-09).

REFERENCES

Clary D.O. & Wolstenholme D.R. 1985: The mitochondrial DNA molecule of Drosophila yakuba). Nucleotide sequence, gene organization and genetic code. *J. Molec. Evol.* **22**: 252–271.

Dirickx H.G. 1994: *Atlas des Diptères Syrphides de la Région Méditerranéenne*. Institut Royal des Sciences Naturelles de Belgique, Bruxelles, 317 pp.

Dirickx H.G. 1998: *Catalogue Synonymique et Géographique des Syrphidae (Diptera) de la Région Afrotropicale*. Museum d'Histoire Naturelle, Genéve, 187 pp.

Dixon T.J. 1960: Key to and descriptions of the third instar larvae of some species of Syrphidae (Diptera) occurring in Britain. *Trans. R. Entomol. Soc. London* **112**: 345–379.

Folmer O., Black M.B., Hoch W., Lutz R.A. & Vrijechock R.C. 1994: DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molec. Mar. Biol. Biotech.* **3**: 294–291.

Goloboff P. 1993: NONA version 2.0. Software available at www.cladistics.com.

Hartley J.C. 1961: A taxonomic account of the larvae of some British Syrphidae. *Proc. R. Entomol. Soc. London* **33**: 505–573.

Hippa H., Nielsen T.R. & Steenis J. 2001: The West Palaearctic species of the genus Eristalis Latreille (Diptera: Syrphidae). *Norw. J. Entomol.* **48**: 289–327.

Kanervo E. 1938: Zur Systematic und Phylogenie der westpaläarktischen Eristalis-arten (Dipt.: Syrphidae) mit einer Revision derjenigen Finnlands. *Ann. Univ. Turk.* **6**: 1–54.

Klein-Krautheim F. 1936: Beitrag zur Kenntnis der Eristalinen-Larven und Puppen (Syrphidae, Diptera). *Stettin. Entomol. Ztg.* **97**: 259–270.

Knüttel H. & Lunau K. 1997: Farbige Augen bei Insekten. *Mitt. Dtsch. Ges. Allg. Angew. Entomol.* **11**: 587–590.

Marcos-García Mª A. & Pérez-Bañón C. 2001: Immature stages, morphology and feeding behaviour of the saprophylic syrphids Copestylum taumalipanum and C. lentum (Diptera: Syrphidae). *Eur. J. Entomol.* **98**: 375–385.

Meigen J.W. 1822: *Systematische Beschreibung der bekannten europäischen zweiflügligen Insekten*. Vol. 3, x + 416 pp., pls. 22–32. Hamm.

Metcalf C.L. 1913: The Syrphidae of Ohio. *Ohio Biol. Surv. Bull.* **1**:1–317.

Mik J. 1897. Einige Bemerkungen zur Dipteren-Familie der Syrphiden. *Wien. Entomol. Ztg.* **16**: 113–119.

Pérez-Bañón C., Rotheray G., Hancock G., Marcos-García Ma.A. & Zumbado M.A. 2003: Immature stages and breeding sites of some Neotropical saprophagous syrphids (Diptera: Syrphidae). *Ann. Entomol. Soc. Am.* (in press).

Peck L.V. 1988: Family Syrphidae. In: Soós Á. (ed.): *Catalogue of Palaearctic Diptera. Vol 8. Syrphidae-Conopidae*. Elsevier, Budapest, pp. 11–230.

Roberts M.J. 1970: The structure of the mouthparts of syrphid larvae (Diptera) in relation to feeding habits. *Acta Zool.* **51**: 43–65.

Rondani C. 1845: Ordinamento sistematico dei generi italiani degli insetti ditteri. *Nuovi Ann. Sci. Nat. Bologna* **2**: 443–59.

Rotheray G.E. 1991: Larval stages of 17 rare poorly known British hoverflies (Diptera: Syrphidae). *J. Nat. Hist.* **25**: 945–969.

Rotheray G.E. 1993: *Colour Guide to Hoverfly Larvae (Diptera: Syrphidae) in Britain and Europe*. Dipterist Digest No. 9. Derek Whiteley, Sheffield, 156 pp.

ROTHERAY G.E. & GILBERT F.S. 1999: Phylogeny of Palaearctic Syrphidae: evidence from larval stages. *Zool. J. Linn. Soc.* **127**: 1–112.

SACK P. 1928–1932: 31 Syrphidae. In: Linder E. (ed.): *Die Fliegen der Paläarktischen Region*. Vol. 4. E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp. 241–288.

SHATALKIN A.I. 1971: A taxonomic analysis of the hoverflies (Diptera: Syrphidae) II. *Entomol. Rev. Wash.* **54**: 127–134.

SHIRAKI T. 1930: Die Syrphiden des Japanischen Kaiserreichs, mit Berrücksichtigung benachbarter Gebiete. *Mem. Fac. Agric. Taihoku Imp. Univ.* **1**: 1–446.

SIMON C., FRATI F., BECKENBACH A., CRESPI B., LIU H. & FLOOK P. 1994: Evolution, weighting and phylogenetic utility of mitochondrial gene-sequences and a compilation of conserved polymerase chain-reaction primers. *Ann. Entomol. Soc. Am.* **87**: 651–701.

SMITH K.G.V. & VOCKEROTH J.R. 1980: Family Syrphidae. In: Crosskey R.W. (ed.): *Catalogue of the Diptera of the Afrotropical Region*. British Museum (Natural History), London, pp. 488–510.

SPEIGHT M.C.D. 2001: Species accounts of European Syrphidae (Diptera), 2001. In: Speight M.C.D., Castella E., Obrdlik P. & Ball S. (eds): *Syrph the Net, the Database of European Syrphidae*, Vol. 27, Syrph the Net publications, Dublin, 281 pp.

THOMPSON F.C. 1972: A contribution to a generic revision of the Neotropical Milesiinae (Diptera: Syrphidae). *Archiv. Zool. S. Paulo* **23**: 73–215.

THOMPSON F.C. 1997: Revision of the Eristalis flower flies (Diptera: Syrphidae) of the Americas south of the United States. *Proc. Entomol. Soc. Wash.* **99**: 209–237.

THOMPSON F.C. 2002: *Syrphidae. Biosystematic Database of World Diptera*. http://www.sel.barc.usda.gov/names, April 2002.

THOMPSON F.C. & ROTHERAY G. 1998: Family Syrphidae. In: Papp L. & Darvas B. (eds): *Contributions to a Manual of Palaearctic Diptera (with Special Reference to Flies of Economic Importance)*. Vol. 3, Higher Brachycera. Science Herald, Budapest, pp. 81–139.

VERLINDEN L. 1994: *Faune de Belgique. Syrphides (Syrphidae)*. Institut Royal des Sciences Naturelles de Belgique, Bruxelles, 289 pp.

Received August 14, 2002; revised January 9, 2003; accepted March 18, 2003