

## Biology of *Eupales ulema* (Germar, 1813) and its taxonomic placement among Eumolpinae (Coleoptera, Chrysomelidae)

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**Résumé.** — *Eupales* Lefèvre est un genre d'eumolpine monospécifique du Sud-Est de l'Europe, principalement de la Hongrie méridionale, de la péninsule des Balkans (Grèce, Bulgarie, Roumanie, ex-Yougoslavie) et de la Turquie. CROWSON (1955) l'a considéré comme un membre primitif de la sous-famille Eumolpinae, partageant des éléments communs avec *Stenomela* et *Hornius* en Amérique du Sud, et avec *Macrolema* et *Spilopyra* en Australie. REID (2000) a décrit ces caractères communs comme étant plésiomorphiques et a exclu *Eupales* des Spilopyrinae, qui est une sous-famille créée par Reid lui-même, pour y inclure *Stenomela*, *Hornius*, *Spilopyra*, *Macrolema*, *Richmondia*, *Cheiloxena*, et *Bohumiljanina*. (d'où, dans cet article, ces genres membres des Spilopyrinae/Spilopyrini seront référés en tant que spilopyrines). Dans ce travail, quelques nouvelles observations sur la biologie et le développement des *Eupales* sont citées, et sur la base des données, à présent disponibles, la position systématique de ce genre dans la classification des Eumolpinae est discutée. Le nom générique *Pales* fut donné par DEJEAN, en 1835, dans la troisième édition de son catalogue, puis par REDTENBACHER, en 1858, et le nom *Eupales* par LEFÈVRE en 1885. Si l'on respecte la priorité, le genre devrait être cité comme *Pales*. Mais le nom générique est pré-occupé pour un Diptère, *Pales* Robineau-Desvoidy, 1830. D'où le nom alternatif *Eupales* adopté ici.

**Summary.** — CROWSON (1955) grouped *Eupales* with spilopyrins. This communication presents observations, which do not support this grouping, though the concept of primitiveness of *Eupales* among Eumolpinae is supported. It has been suggested that *Eupales* be placed in a tribe of its own, the Eupalini.

### Introduction

*Eupales*, Lefèvre is a monospecific eumolpine genus from South-East Europe, mostly Hungary, the Balkan Peninsula (i.e. Greece, Bulgaria, Romania, former Yugoslavia) and

Turkey. CROWSON (1955) regarded it as a primitive member of the subfamily Eumolpinae, sharing several common features with *Stenomela* and *Hornius* in South America, and *Macrolema* and *Spilopyra* in Australia. REID (2000) described these common characters as plesiomorphic, and excluded *Eupales* from Spilopyrinae, which is a subfamily, created by Reid himself, to include *Stenomela*, *Hornius*, *Spilopyra*, *Macrolema*, *Richmondia*, *Cheiloxena* and *Bohumiljania*. Henceforth in this communication these member genera of Spilopyrinae/Spilopyrini will be referred to as spilopyrins. In this paper some new observations on the biology, life-history and results of DNA studies on *Eupales* are being reported, and on the basis of the information, now available, the systematic position of this genus in the classification of Eumolpinae is discussed. The generic name *Pales* was given by DEJEAN in 1835, in the third edition of his catalogue, then by REDTENBACHER, 1858, and the name *Eupales* by LEFÈVRE in 1885. Respecting priority, the genus should be referred to as *Pales*, but this generic name is preoccupied for a fly, *Pales* Robineau-Desvoidy, 1830. Hence the alternative name *Eupales* is used here.

### Material and methods

*Eupales ulema* (Germar, 1813), described first as a *Colaspis*, has been studied from the standpoint of biology and life-history in Hungary by making observations both in nature and in culture jars/Petri dishes. For the study of male and female genitalia dry Greek adults of this species, provided by Serge Doguet and Ph. Ponel, were dissected. Genetic data for *E. ulema* and several other eumolpines were available from the recent phylogenetic study of this group of beetles (GÓMEZ-ZURITA *et al.*, 2005). Average genetic distances and their standard error between sequences and selected groups of sequences were estimated using the software MEGA version 3.0 (KUMAR *et al.*, 2004). Divergence estimates were calculated using pairwise deletion of gapped and missing data and applying the TAMURA and NEI (1993) model of sequence evolution, including both transitions and transversion and assuming uniform rates of change among sites. The corresponding standard error measures were computed by bootstrap analysis, with 500 pseudoreplicates.

### Observations

#### (i) Food plant

*E. ulema* has been reported from two species of *Cornus*, viz. *Cornus mas* L. and *C. sanguinea* L. (Cornaceae), and also on other plants like *Pyrus* spp. (Rosaceae) (JACQUELIN DU VAL, 1886). In Hungary, according to Károly Vig's observations, the insect seems to be confined to *Cornus mas*, as it could not be found on other species of *Cornus*, and on any other plant species.

#### (ii) Some features of the adult anatomy

The adult of *E. ulema*, has an elongated oval body (pl. 1, fig. 1). Main adult features of this species have been described by CHAPUIS (1874).

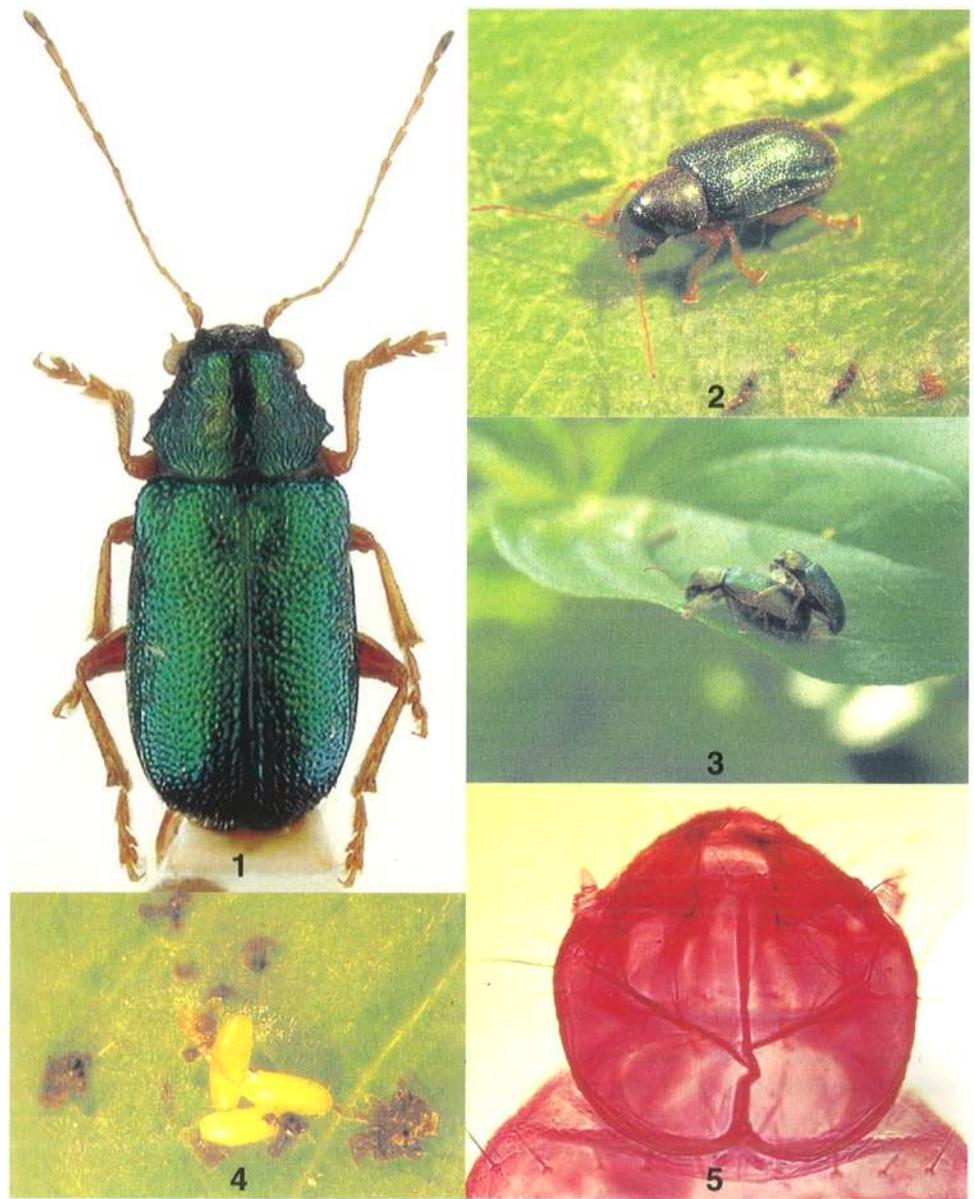


Plate 1 : *Eupales ulema* (Germar, 1813). — Adult male (x17). — Adult female on its host plant, *Cornus mas* L. — Copulating pair. — Eggs on a leaf of *Cornus*. — Photograph of a larva of *E. ulema*, dorsal view of anterior region.

(1 : photo Zoia ; 2 & 4 : photo Vig and Dankovics ; 3 : photo Kisbenedek ; 5 : photo Vig).

The wing venation in *E. ulema* (pl. 3, fig. 1) shows the typical eumolpine condition, but the mediocubital fleck is weakly developed (JOLIVET, 1957-1959).

The aedeagus of *Eupales* (pl. 2, fig. 2) presents the following seemingly primitive features, shared with spilopyrins :

- (a) Ventral curvature of the aedeagus is only moderate, and not as deep as in higher Eumolpinae.
- (b) There is no deep constriction on the dorsal side between the basal hood and the aedeagus proper.

The aedeagus of *Eupales* (pl. 2, fig. 2) presents the following seemingly primitive features, shared with higher eumolpines (for details on the aedeagal apparatus in spilopyrins, see VERMA and JOLIVET, 2004).

- (a) Differentiation of the basal hood is better marked than in spilopyrins.
- (b) The second spiculum or the tegmen, with its arms, is confined to the anteroposterior region of the ventrally displaced basal orifice, i.e. the arms do not extend beyond the ventroposterior lip of the basal orifice.
- (c) The tegmen is fixed in its position, and cannot be moved along the aedeagus, as has been noted also by Zoia (personal communication, 2002). This situation suggests that the articulation between the forked posterior end of the tegmen and the ventroposterior edge of the basal orifice is through a limited area of arthroal membrane. In contrast, in *Cheiloxena*, as pointed out by Zoia, and in *Bohumiljanina*, as seen by Verma, the tegmen readily moves along the aedeagus, suggesting the presence of an ample arthroal membrane between the tegmen and the ventroposterior edge of the basal orifice, an almost "galerucine"-like condition.

The spermathecal complex of *E. ulema* (pl. 3, fig. 2) presents a special feature. The proximal part of the spermathecal capsule, receiving the duct of the gland and giving out the spermathecal duct, is bifurcated, one part for the gland duct and the other for the spermathecal duct. None of the figures in SUZUKI (1988) shows this feature. At this moment this feature in *E. ulema* appears autapomorphic.

### (iii) Life-history

Copulation occurs in the typical riding mode, common among leaf beetles (pl. 1, fig. 3).

In cultures, *E. ulema* females lay elongated eggs on leaves of the host plant or on the walls of the Petri dish (pl. 1, fig. 4). The incubation period is surprisingly long; it takes on average one month at room temperature for the eggs to hatch. Many eggs are infected with entomophagous fungi. It is the same when you try to rear *Aulacoscelis* and *Megascelis* under laboratory conditions.

The neonate larvae are white-yellowish in colour (pl. 1, fig. 5). Like typical eumolpines, larvae are fully blind, not even with a spot where there should be stemmata. These larvae do not feed on leaves. When transferred to *Cornus* growing in a plot, they penetrate into soil quickly. They feed in soil on roots only. There could not be found any polyphagy on plant roots. Most likely they feed normally only on roots of *Cornus mas*,

which is also the food plant for the adult. Probably in the wild, the eggs are laid in the soil at the foot of the host plant. The neonate larva does not show any caudal abdominal shield, which is presumably a plesiomorphic feature shared by spilopyrins and galerucines among some others.

#### (iv) DNA analysis

*Eupales ulema* has been recently included in a molecular phylogenetic study to address the interrelationship of the subfamilies Spilopyrinae and Eumolpinae (GÓMEZ-ZURITA *et al.*, 2005). The study, based on the parsimony analysis of about 3000 bp of three ribosomal markers (partial sequences of the 16S, 18S and 28S rDNAs) and several morphological characters, has demonstrated the basal position of *Eupales* in a monophyletic Eumolpinae clade, separated from the Spilopyrinae clade including samples of the genera *Bohumiljanina*, *Hornius*, *Spilopyra* and *Stenomela* (pl. 4).

A complementary approach to the phylogenetic analyses in GÓMEZ-ZURITA *et al.* (2005) consists in the study of genetic distances among groups of DNA sequences. The available genetic data was used to estimate average genetic distances within and between groups of sequences, including the Eumolpinae, the Spilopyrinae, several outgroups from other leaf beetle subfamilies, and their respective distances with *E. ulema* (Table 1). Excluding the outgroups, the lowest genetic divergence of *E. ulema* was  $0.0375 \pm 0.0037$  with *Stenomela pallida* (Spilopyrinae), and the highest,  $0.0613 \pm 0.0046$ , was obtained with *Pachnephorus impressus* (Eumolpinae). The inclusion of *E. ulema* in either Spilopyrinae or Eumolpinae had an insignificant effect on the corresponding divergence estimates, except for the average divergence within Spilopyrinae, increasing from 0.0279 to 0.0328.

Table 1  
Average genetic distances, applying the Tamura and Nei (1993) correction, between groups of ribosomal DNA sequences in the Eumolpinae and related taxa.

	outgroup	Spilopyrinae	Eumolpinae
outgroup	$0.0416 \pm 0.0024$		
Spilopyrinae	$0.0435 \pm 0.0028$	$0.0279 \pm 0.0027$	
Eumolpinae	$0.0509 \pm 0.0029$	$0.0477 \pm 0.0029$	$0.0408 \pm 0.0025$
<i>Eupales ulema</i>	$0.0443 \pm 0.0034$	$0.0403 \pm 0.0035$	$0.0457 \pm 0.0034$

#### Discussion

CHAPUIS (1874) described in good detail the external features of adult *Eupales*, and regarded the beetle as "remarkable", perhaps because of its primitive features, such as non-bifid claws. As mentioned above, CROWSON (1955) clearly stated that this eumolpine is primitive, and shares a number of primitive features with spilopyrins. REID (2000) adhered to this view and regarded *E. ulema* as a "basal lineage of Eumolpinae".

From our current point of view grouping of *Eupales* with spilopyrins, as suggested by CROWSON (1955), is not acceptable. Common features, which we regard as plesiomorphic,

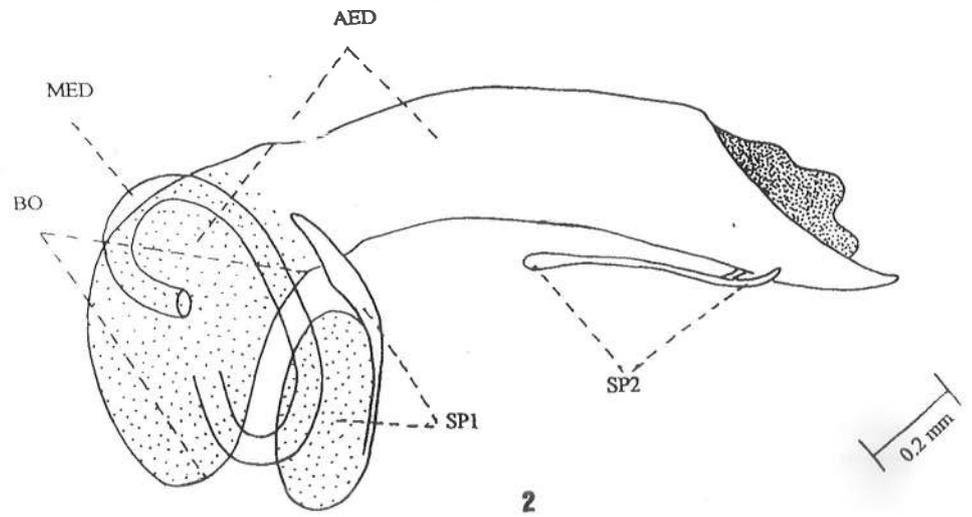
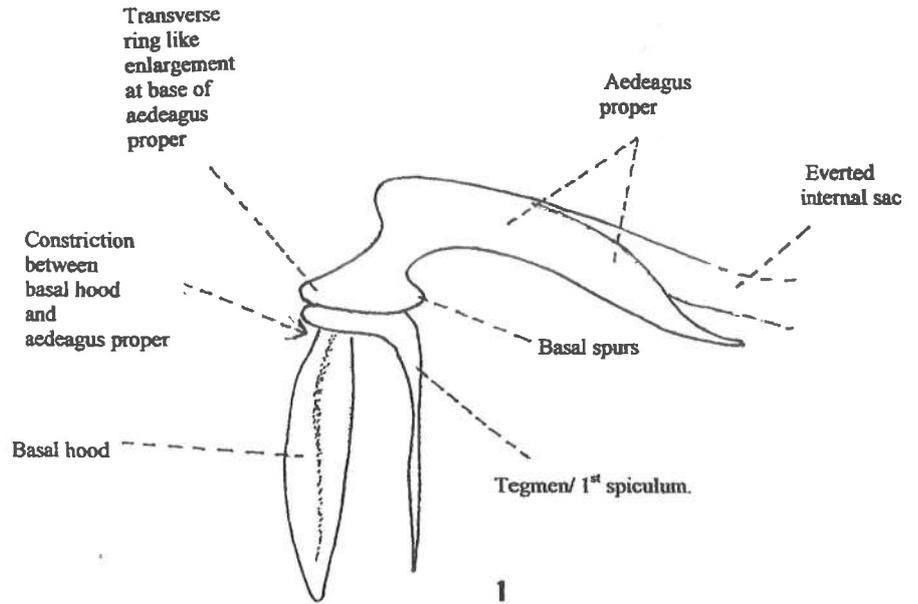


Plate 2 : 1. Aedeagus of a classical eumolpine, *Colaspis* sp. — 2. Aedeagal apparatus of *E. ulema*, in lateral view. AED = aedeagus, BO = basal orifice, MED = median ejaculatory duct, SP1 = first spiculum or tegmen, SP2 = second spiculum. Sparsely dotted parts are less well sclerotised and relatively soft.

(from VERMA & JOLIVET, 2004).

should not be taken as a pointer to monophyly. The aedeagal features of *Eupales* are primitive, but not as primitive as those of spilopyrins. The autapomorphic characteristics of the spermatheca of *E. ulema*, i.e. the spermathecal capsule presenting a bifurcation in its proximal part, also separate this taxon from spilopyrins.

Larvae of spilopyrins are exposed feeders on leaves of the host plant, and those of typical eumolpines enter soil to feed on roots. Our observations have shown that the neonate larvae of *Eupales* enter soil, and do not feed on leaves. Larvae of Eumolpinae are generally blind, as they do not have ocelli (BOVING & CRAIGHEAD, 1930; PETERSON, 1951; STEHR, 1991). However, REID (2000) has recorded two ocelli on each side in larvae of Eumolpini and two stemmata have been observed in the larva of *Platycornus* Chevrolat (Verma, K. K., unpublished). On the contrary, in spilopyrin larvae there are five ocelli on each side of the head (VERMA & JOLIVET, 2004). In contrast the larva of *Eupales* is blind, as in higher Eumolpinae. In the larva of *E. ulema* there is lack of the hard supra-anal dorsal abdominal shield, which is present in larvae of spilopyrins (VERMA & JOLIVET, 2004).

From a biogeographical perspective, spilopyrins occur in the Southern Hemisphere in lands of the Gondwanian origin (REID, 2000; VERMA & JOLIVET, 2004). But *E. ulema* is found in a land mass north of the palaeogeological Sea Tethys.

The only available molecular phylogenetic study including samples of *E. ulema* together with several other Eumolpinae and Spilopyrinae does not place *Eupales* within the monophyletic Spilopyrinae, but at the base of the Eumolpinae clade instead (GÓMEZ-ZURITA *et al.*, 2005). This result is consistent with the views exposed above suggesting in first place that *E. ulema* is not a spilopyrin and that it is an extant lineage of the earliest radiation of the Eumolpinae. However, it is intriguing that ignoring the tree topology and studying the plain genetic distances between pairs of sequences, the lowest genetic divergence of the *Eupales* ribosomal data corresponds in fact to that with the spilopyrin *Stenomela pallida*, and although the differences are only in the order of a 12%, the *Eupales* sequences are on average more similar to those of the sampled Spilopyrinae than to the Eumolpinae. These results could be due to the ancient separation of the *Eupales* lineage from the main Eumolpinae stem, little younger than the branching off that would lead to the Spilopyrinae lineage, therefore almost equally divergent from extant members of the two clades. Alternatively, although the distance estimates have been corrected with the TAMURA & NEI (1993) transformation, these could be in fact inaccurate estimates because of the effects of homoplasy and/or heterogeneities in the rates of evolution across sites and taxa. Finally, the topology obtained in GÓMEZ-ZURITA *et al.* (2005), with *E. ulema* monophyletic with the other Eumolpinae, could be an artifact resulting from the absence in the analyses of representatives of other subfamilies of leaf beetles, potential sisters to the Eumolpinae, e.g. Cryptocephalinae and related taxa (REID, 1995).

Nevertheless, in the absence of specific analysis to refute the proposed alternatives, we adhere to the results pointed out by the current molecular phylogenetic evidence, i.e. *E. ulema* is the sister to modern Eumolpinae and the result of an ancient ramification of the Eumolpinae lineage, as suggested by the high genetic distances registered.

In brief, there is not enough reason for grouping *Eupales* with spilopyrins.

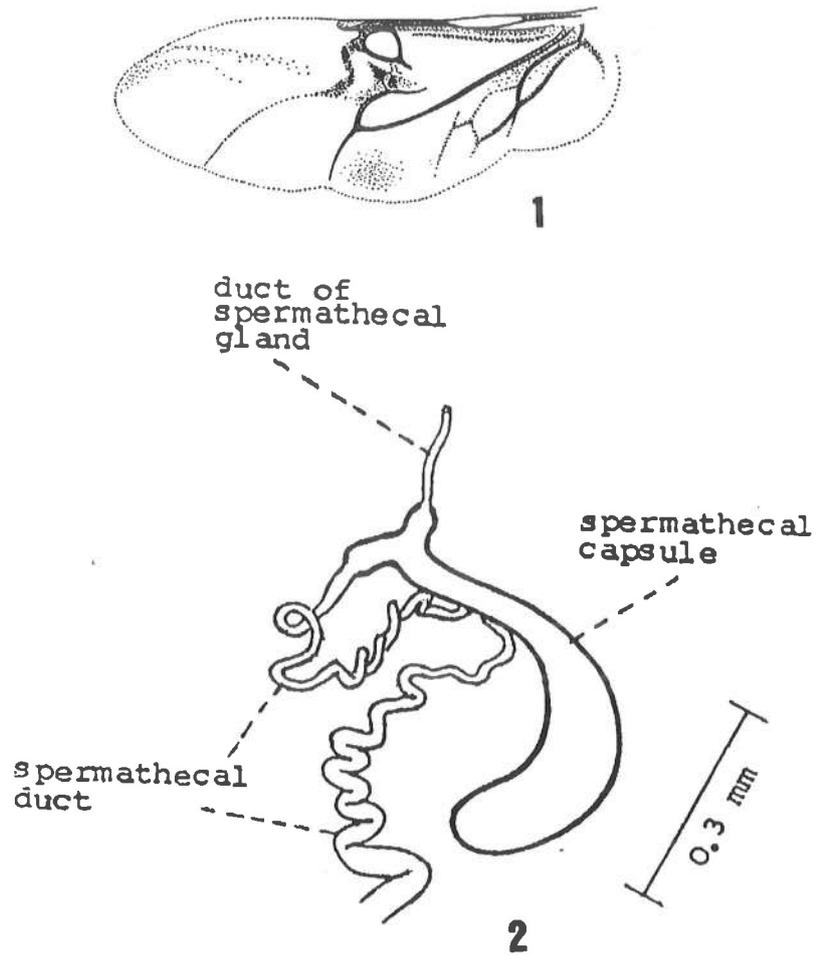


Plate 3 : 1. Wing of *Eupales ulema*, typically eumolpine. — 2. Spermathecal complex of *E. ulema*. (1 : from JOLIVET, 1957 ; 2 : from VERMA & JOLIVET, 2004).

On the other hand, primitiveness of *Eupales* cannot be ignored. In addition to the primitive feature, pointed out by CROWSON (1955), REID (2000) has mentioned some additional plesiomorphic features, which *Eupales* shares with spilopyrins, viz. “tibial spurs, a long prosternum anterior to coxae, no lateral projections on the prosternal process, only vaguely defined subcubital fleck (JOLIVET, 1959), non-telescopic ovipositor, kotpresse represented by chitinpolster only, and simple penis....”. Primitive features of the aedeagus have been mentioned in this communication (*vide supra*).

In *Eupales* some features, e.g. the spermathecal capsule presenting bifurcation in its proximal part, seem to be autapomorphic.

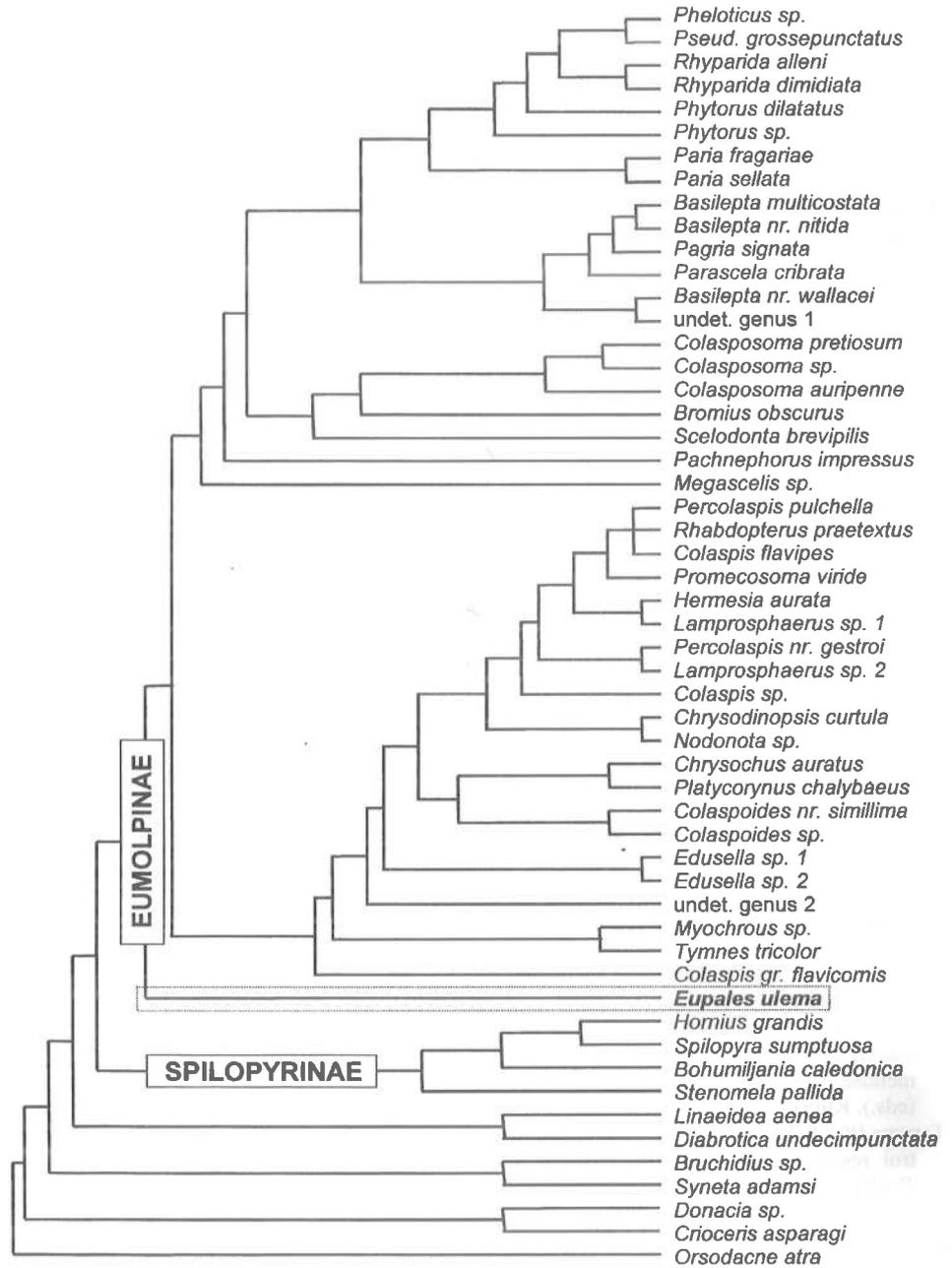


Plate 4 : Most parsimonious phylogenetic tree of morphological and ribosomal DNA sequence data for the Eumolpinae and Spilopyrinae (GÓMEZ-ZURITA *et al.*, 2005). The placement of *Eupales ulema*, in an intermediate position between Eumolpinae and Spilopyrinae, rendering a monophyletic Eumolpinae clade, is indicated with a dotted box.

Which position should be ascribed to *Eupales* in the classification of Eumolpinae in the light of the discussion above? SEENO & WILCOX (1982) placed *Eupales* under the tribe Eumolpini. However, in view of its primitive features, but showing considerable differences from spilopyrins, our opinion is that *Eupales* may be placed in a tribe of its own, and be regarded as representing, in words of REID (2000), "a basal lineage of Eumolpinae". Some eumolpines, discovered and studied in the future, may join this tribe. We suggest the name Eupalini for the currently monogeneric and monospecific tribe including *Eupales ulema*.

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