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New data on European *Astragalus*-feeding *Bruchidius*, with the description of a new species from Southern Italy
(Coleoptera: Bruchidae: Bruchinae)

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ABSTRACT. *Bruchidius bernardi* n. sp., a seed-beetle feeding in *Astragalus depressus* pods, is described from southern Italy. A new synonymy is proposed: *Bruchidius myobromae* (MOTSCHULSKY, 1874) (= *Mylabris virgata* var. *scutulata* BAUDI, 1890). The host plant of *Bruchidius poecilus* (GERMAR) in the same area is identified for the first time as *Astragalus contortuplicatus*. DNA of various related *Bruchidius* species from southern France and Italy was analysed in order to assess the phylogenetic relationships between them. Parsimony analysis of the partial 12S rRNA gene of eight species yielded a phylogenetic tree showing that *B. bernardi* and other *Astragalus* species belong to the *Bruchidius astragali*-group, whereas *B. poecilus* clusters with *Bruchidius* species feeding on various Trifolieae (*Trifolium* and *Medicago*). More comprehensive data are needed in order to establish the phylogeny of the group and related species.

Key words: entomology, taxonomy, phylogeny, new species, host plant, legumes, *Astragalus*, Coleoptera, Bruchidae, *Bruchidius*.

INTRODUCTION

With about 2500 species in Eurasia and the Americas, *Astragalus*, a member of the tribe Galegeae (BRONN), subtribe Astragalinae (ADANS.), is the world's largest genus of vascular plants; diversification rates in the „Astragalean clade” (*Astragalus*, *Oxytropis* and a few close relatives) seem to be significantly higher

than in other Angiosperms (SANDERSON & WOJCIECHOWSKI 1996; WOJCIECHOWSKI, SANDERSON & JER-MING HU 1999). A central hypothesis in the field of insect speciation is that diversification of vascular plants has to do with the diversification of phytophagous insects, the latter closely following the appearance of new plant species. Insects themselves may probably be one of the driving forces of plant diversification, but their precise importance is virtually unknown. The case under study is of particular interest in that respect because Galegeae constitute a highly diversified and relatively well defined group of plants, with a number of identified bruchids feeding on their seeds.

In 1985, BOROWIEC defined within the genus *Bruchidius* the *astragali*-species group for insects morphologically close to *Bruchidius astragali* (BOHEMAN, 1829) and feeding (or supposed to feed) in the seeds of various *Astragalus* species. In addition to *B. astragali*, the following species were originally included in the group: *B. atbasaricus* (LUKJANOVITCH & TER-MINASSIAN), *B. lucifugus* (BOHEMAN), *B. marginalis* (F.), *B. myobromae* (MOTSCHULSKY), *B. scutulatus* (BAUDI), *B. tragacanthae* (OLIVIER), *B. virgatoides* (LUKJANOVITCH & TER-MINASSIAN), *B. virgatus* (FAHRAEUS). In 1988, BOROWIEC added *B. brignolii* ZAMPETTI to the group. All these species share the following set of characters: body oval; antennae strongly sexually dimorphic; elytral base without tubercle, or with a very small one; mucro shorter than lateral teeth of corona; edeagus rather slim, internal sac with small spines; lateral lobes of parameres flat, with a feebly modified apex; base of parameres with a carina. Of the ten species included by BOROWIEC in the *astragali*-group, two, namely *atbasaricus* and *brignolii*, were treated as synonyms by ANTON (1998), while we propose *scutulatus* as synonym (see chapter Results). Of the remaining species, *lucifugus*, *tragacanthae* and *virgatoides* were actually not reared from *Astragalus* seeds, but were supposed to feed in them, or were caught on *Astragalus* plants.

Recently *B. myobromae* was listed for S. Tirol (Italy) by KAHLEN (1987, erroneously as *virgatus*). In June 2001, with the help of M. KAHLEN from Museum Ferdinandeum, Innsbruck (Austria), specimens were found at the cited locality on blooming *A. exscapus*; one month later adults of *B. myobromae* emerged from the seeds of the same host plant.

A number of other *Bruchidius* species are *Astragalus*-feeders: *B. caninus* (KRAATZ), *B. gombo* (PEYERIMHOFF), *B. leprieuri* (JACQUET), *B. longulus* (SCHILSKY), *B. plagiatus* (REICHE & SAULCY), *B. poecilus* (GERMAR), *B. richteri* LUKJANOVITCH & TER-MINASSIAN and *B. varipes* (BOHEMAN) (table 1). Among these, *B. longulus* and *B. poecilus* were included by BOROWIEC (1988) in the *Bruchidius tibialis* and *varius*-groups, respectively. According to DECELLE & LODOS (1989), *B. sivasensis* (ZAMPETTI) and *B. talyshensis* TER-MINASSIAN (under the name *kurdicus* DECELLE, nomen nudum) were caught on *Astragalus* plants. Here we assign the species *caninus*, *leprieuri*, *richteri*, *sivasensis*, *talyshensis* and *varipes* as members of the *astragali* group, and *gombo* as a member of the *halodendri* group. It is worth mentioning that a few other *Bruchidius* species, known to feed in the seeds of different Leguminosae, are sometimes associated with *Astragalus*: *B. bimaculatus*

(OLIVIER), *B. cisti* (F.), *B. villosus* (FABRICIUS), incorrectly under the name *fasciatus* (OLIVIER), *B. nanus* (GERMAR) (CAILLOL 1954; DE LUCA 1962; ZACHER 1951). These doubtful records may either result from misidentification, or misinterpretation of biological data and need confirmation.

It may be added that in the New World, a small number of species (less than 10) in the genus *Acanthoscelides* have been reported as feeding in various species of *Astragalus* (JOHNSON 1970).

In 2002 and 2003, four species of *Bruchidius* were reared from *Astragalus* pods collected in Southern France, Corsica and Italy. Among these, one species reared from *Astragalus depressus* pods proved to be new to science and is

Tab. 1: Insect-host plant relationships between *Bruchidius* and *Astragalus*.

Bruchid species	<i>Astragalus</i> host species	References ^c
<i>B. astragali</i>	<i>A. mollis, ponticus, testiculatus</i>	1, 8, 11, 19
<i>B. bernardi</i>	<i>A. depressus</i>	present
<i>B. caninus</i> ^a	<i>A. boeticus, chlorostegius, hamosus, tragacantha</i> ^b	6, 13, 14, 15
<i>B. gombo</i>	<i>A. gombo</i>	18
<i>B. leprieuri</i>	<i>A. caprinus, pulcher, A. sp.</i>	10, 12, 16
<i>B. longulus</i>	<i>A. monspessulamus</i>	9
<i>B. lucifugus</i>	unknown	-
<i>B. marginalis</i>	<i>A. glycyphyllos, hamosus, monspessulamus, vesicarius</i>	4, 6, 13, 15, 16
<i>B. myobromae</i>	<i>A. exscapus, utriger</i>	22, present
<i>B. plagiatas</i>	<i>A. caraganae, macrocarpus</i>	5, 20
<i>B. poecilus</i>	<i>A. contortuplicatus</i>	present
<i>B. richteri</i>	<i>A. sp.</i>	21
<i>B. sivasensis</i>	probably <i>A. sp.</i>	17
<i>B. talyshensis</i>	probably <i>A. gummifera</i>	17
<i>B. tragacanthae</i>	probably <i>A. sp.</i>	2
<i>B. varipes</i>	<i>A. ponticus</i>	3 ^d
<i>B. virgatoides</i>	unknown	-
<i>B. virgatus</i>	<i>A. monspessulamus, sieverstanus, ?pinetorum, "fabago"</i>	7, 11, 20

^a A variable species or a group of species. There are also reports of *B. caninus* feeding in pods of *Oxytropis*, a genus very close to *Astragalus* in the tribe Galegeae.

^b *A. massiliensis*, a synonym of *A. tragacantha*, was reported as host of *B. marginalis* by DELOBEL & DELOBEL (2003); the specimens feeding in *A. massiliensis* seeds in Corsica seem to be more closely related with *B. caninus* than with *B. marginalis*.

^c Numbers in this column refer to the following articles: 1: ABDUL-RASSOUL *et al.* (1986); 2: ANTON (1998a); 3: BRANDL (1981); 4: CAILLOL (1954); 5: CALDERON (1962); 6: DELOBEL & DELOBEL (2003); 7: DE LUCA (1962); 8: FAHRAEUS (1839); 9: HOFFMANN (1945); 10: JACQUET (1886); 11: LUKJANOVITCH & TER-MINASSIAN (1957); 12: NORMAND (1937); 13: PARKER (1957); 14: PEYERIMHOFF (1926); 15: SCHILSKY (1905); 16: ZACHER (1952); 17: DECELLE & LODOS (1989); 18: PEYERIMHOFF (1915); 19: TER-MINASSIAN (1954); 20: KARAPETIAN (1985); 21: IABLOKOV-KHNZORIAN (1959); 22: MOTSCHULSKY (1874).

^d under *B. astragali* (see ANTON 1998b)

described here. Also, pods of a second species, *A. contortuplicatus*, yielded a species hitherto without known host plant, *B. poecilus*. The various specimens collected have given us the opportunity to assess relationships among *Astragalus*-feeding species from the West Mediterranean area. Other species listed in Tab.1 were not available for analysis, most of them having an East Mediterranean or Central Asian distribution.

MATERIAL AND METHODS

A. depressus is a short-stemmed legume that grows in middle to high altitude meadows of Southern Europe and North Africa. A sample of 88 ripe or nearly ripe pods were collected in the vicinity of the Gasperi refuge, on the slopes of Monte Pollino (Basilicata region, Italy), about 1535m above sea level, in late June 2003. Pods were kept in aerated plastic bags until emergence of adults. A total of 10 adult specimens emerged, one of which was used for DNA extraction in order to assess the relationships of the species with other *Astragalus*-feeding species. Other specimens used for DNA extraction and analysis are shown in Tab. 2. Among these, adults of *B. poecilus* were obtained from infested pods of *A. contortuplicatus* collected near San Severino Lucano, 1150m a.s.l. (Basilicata region, Italy) in June 2003. Four *Bruchidius* species not belonging to the *astra-*

Tab. 2: Material examined in this study.

Genus and species	Locality	Host plant ^a (family, tribe) ^b
<i>Bruchidius</i>		
<i>bernardi</i> (DELOBEL & ANTON, 2004)	Basilicata (Italy)	<i>Astragalus depressus</i> (F, Gal)
<i>bimaculatus</i> (OLIVIER, 1795)	Haute Corse (France)	<i>Medicago marina</i> (F, Tri)
<i>caninus</i> (KRAATZ, 1869)	Haute Corse (France)	<i>Astragalus hamosus</i> (F, Gal)
<i>fulvicornis</i> (MOTSCHULSKY, 1874) ^c	Corse du Sud (France)	<i>Trifolium vesiculosum</i> (F, Tri)
<i>marginalis</i> (FABRICIUS, 1776)	Gard (France)	<i>Astr. monspessulanus</i> (F, Gal)
<i>nanus</i> (GERMAR, 1824)	Basilicata (Italy)	<i>Medicago orbicularis</i> (F, Tri)
<i>poecilus</i> (GERMAR, 1824)	Basilicata (Italy)	<i>Astragalus contortuplicatus</i> (F, Gal)
<i>varipictus</i> (MOTSCHULSKY, 1874)	Haute Corse (France)	<i>Medicago murex</i> (F, Tri)
<i>Pachymerus</i>		
<i>cardo</i> (FAHRAEUS, 1839)	French Guyana	<i>Elaeis</i> sp. (Ar)
<i>Spermophagus</i>		
spec.	Gard (France)	

^a We only figured the host-plant species from which the sequenced individual has been reared.

^b Host-plants systematic was abbreviated as follows: Ar (Arecaceae), F (Fabaceae), Gal (Galegeae), Tri (Trifolieae).

^c sensu BOROWIEC (1988).

gali-group were included in the analysis in order to assess more precisely the position of *B. poecilus*. The choice of these species was based on their proximity (less than 5% divergence) with *B. poecilus* in a distance matrix for 12 rRNA; these four species (*B. bimaculatus*, *B. fulvicornis* sensu BOROWIEC, *B. nanus*, and *B. varipictus*) feed exclusively on members of the Trifolieae tribe.

We used whole individuals for DNA extraction and we followed the protocol for insect tissues recommended by the QIAGEN DNeasy tissue kit (QIAGEN GmbH, Germany), with PBS used. For the amplification of partial sequences of the 12s rRNA gene we used primers (Simon et al. 1994) SR-J-14233 (5'-AAGAGCGAC GGGCGATGTGT-3') and SR-N-14588 (5'-AACTAGGATT AGATACCCTA TTAT -3') with standard PCR conditions. PCR products were purified by using QIAquick PCR purification kit (QIAGEN GmbH, Germany). Both strands were sequenced for all taxa by the Sanger dideoxy method and sequence data were obtained by analyzing samples on an ABI 373 automated sequencer. Sequences were aligned by means of ClustalX (THOMPSON 1997) with default settings.

Parsimony analysis (unweighted) was performed using PAUP* version 4.0b10 (SWOFFORD 2002) and we used *Pachymerus cardo* and *Spermophagus* sp. as outgroup species.

Parsimony trees were obtained by heuristic search option of 100 random addition replicates (with gaps treated as fifth base). Confidence in each node was assessed by bootstrap procedures by using 100 replicates of 100 random-addition replicates each.

RESULTS

SYNONYMY

***Bruchidius myobromae* (MOTSCHULSKY, 1874)**

Bruchus myobromae MOTSCHULSKY, 1874: 212.

Bruchidius myobromae – LUKJANOVITCH & TER-MINASSIAN, 1957: 165.

Mylabris virgata var. *scutulata* BAUDI, 1890: 342, **syn. nov.**

Bruchus virgatus var. *scutulatus* – PIC, 1913: 57.

Bruchidius virgatus var. *scutulatus* – SCHILSKY, 1905: no. 58.

Bruchidius scutulatus – LUKJANOVITCH & TER-MINASSIAN, 1957: 167.

DISTRIBUTION

Italy (S. Tirol: Vinschgau), Ukraine, S. Russia, Kazakhstan (“Turkestan”), Iran.

REMARK

Each male and female type of *B. myobromae*, designed by K.-W. ANTON as lectotype and paralectotype, are preserved in ZMAS, St. Petersburg (Russia); the male type bears two labels: “Ex Myobro / ma utri- / gera Taur.”, “Bruchus /

myobromae / Motsch. / Tauria” (both labels yellowed white, handwritten in ink), the female type “Tauria” only (small red label, handwritten in ink). The male type of *M. virgata* var. *scutulata*, also designed by K.-W. Anton as lectotype, preserved in MRSN, Torino (Italy), has the following labels: “31 / 61”, “Genital / in euparal / Zampetti / 1980” (yellow label, handwritten by Zampetti), “Mylabris virgata / var. scutulata Baudi / Turkestan” (white label, handwritten). All types represent the same species.

DESCRIPTION OF THE NEW SPECIES

***Bruchidius bernardi* DELOBEL & ANTON, n. sp.**

TYPE MATERIAL

Holotype: Male, “Italie – Basilicata 25.06.2003 / Ex gr. Astragalus depressus/ Refuge de Gasperi (1535 m) / A. & B. Delobel coll. Italie 08”, MNHN. Paratypes: 1 male and 8 females, same data as holotype, MNHN and CKWA.



1. *Bruchidius bernardi*: dorsal aspect, male (left) and female

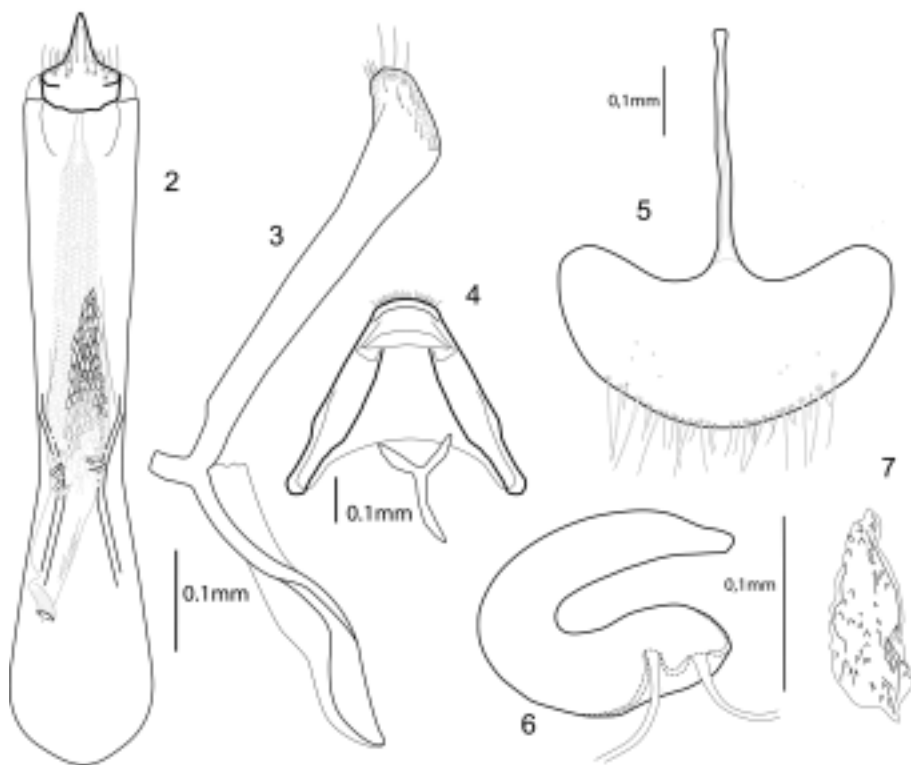
DIAGNOSIS

A small-sized species with completely black integument, uniformly greyish vestiture, sexual dimorphism of antenna, indistinct protuberance with minute denticle at base of elytra, preapical denticle of hind femur reduced to minimum, short mucro at apex of hind tibiae, male genitalia as shown in Figs 2-4.

DESCRIPTION

Length: 2.4-2.5 mm, width: 1.4 mm. Body oblonge-oval (Fig. 1). Integument completely black. Vestiture dense, covering integument nearly completely, recumbent; scutellum and hind edges of pronotum with denser whitish hairs; remaining parts of body with short greyish hairs.

Male: Head short, constricted behind eyes; eyes bulging, maximum head width about 1.5 times width behind eyes; ocular sinus deep; ratio of eye width to minimum distance between eyes about 1.25; postocular lobes reduced to minimum; diffuse carina on frons, vertex with diffuse interocular tubercle. Antenna



2-7, *Bruchidius bernardi*. 2. median lobe, ventral view; 3. lateral lobes and tegminal strut, lateral view; 4. genital segment of male, ventral view; 5. spiculum ventrale of female; 6. spermathecal body; 7. dorsal sclerite of bursa copulatrix

reaching to end of basal quarter third of elytra; antennal segments 1-2 cylindrical, segment 3 subserrate, segments 4-10 serrate, segment 11 oblonge with apex acute; segment 1 about twice as long as segment 2, segment 3 slightly longer and broader than segment 2, segment 4 about 2.5 times as long and twice as wide as segment 3, segments 4-10 becoming steadily wider, segments 8-10 about 1.1 times as long as wide, segment 11 about twice as long as wide.

Pronotum campaniform, about 1.1 times as wide as long, greatest width at base, sides bisinuate, disc feebly convex, barely visible depression near hind edge; punctures on disc somewhat variable, setous; punctures almost touching each other to as wide as puncture diameter; cuticle between punctures partially shiny. Scutellum of moderate size, as long as wide, bifid.

Elytra oblonge, about 1.3 times as long as their combined width; sides nearly parallel at mid third; disc flattened to feebly convex; indistinct basal protuberance at striae 3-4; a minute sharp tooth at base of stria 4; striae on disc deep, punctured; punctures with setae and as wide as striae, distances between punctures on average longer than their diameter; interstriae flat, with micropunctuation and irregular row of flat coarse punctures.

Legs without sexual dimorphism; hind femora moderately incrassate, at their widest 1.7 times wider than mid femora; mesoventral margin without or with minute, barely visible preapical denticle; hind tibiae simple, with carinae reduced to minimum and barely distinguishable, apex of tibiae with mucro short, mucro as long as or shorter than dorsolateral denticles, dorsolateral denticles distinctly shorter than lateral denticle.

Abdomen simple; sternite 1 without area of denser setae; sternite 5 slightly emarginate; pygidium nearly as wide as long, homogeneously convex towards apex.

Genitalia: Median lobe oblonge, ventral valve subtriangular, with acute apical tip and about 8 setae in each basolateral half; apical third of internal sac with numerous square plates, in mid third followed by numerous less sclerotized denticles and transparent microdentate plates (Fig. 2). Lateral lobes apically broadened, separated to about 0.8 of their length, with 3 long setae at medial half of apex; tegminal strut partly membranous, with large median carina (Fig. 3). Spiculum gastrale short, basally irregularly expanded, apically bifurcate, with short projections, Y-shaped (Fig. 4)

Female: Similar to male, but antennal segments shorter and slimmer, segment 3 cylindrical, segment 4 subserrate, segment 4 feebly longer than segment 3, segment 5 about twice as long and wide as segment 4, segments 8-10 as long as wide, sternite 5 not emarginate, pygidium as long as wide and less convex. Genitalia: spiculum ventrale as in Fig. 5; spermathecal body blackish, of almost equal diameter throughout, strongly recurved, with apical diverticulum pointed; opening of spermathecal gland duct basally (Fig. 6); vagina with a single elongated sclerite on dorsal side (Fig. 7); bursa copulatrix without spines or needles.



8. Adult of *Bruchidius bernardi* ready to emerge from an *Astragalus depressus* pod; the ovoid whitish structure above the emergence hole is an hatched egg

AFFINITIES

Bruchidius bernardi is distinguished from the closely related *B. caninus* (KRAATZ, 1869) by having completely black integument, uniformly greyish pubescence, antennal segments more strongly serrate, indistinct elytral protuberance, and numerous transparent plates in apical third of internal sac.

ETYMOLOGY

Named in honour of Bernard DELOBEL, who collected and identified *Astragalus depressus* and *A. contortuplicatus* pods.

BIOLOGY

Type specimens were reared from pods of *Astragalus depressus* L. Translucent eggs (Fig. 8) are laid on the pod when it is still green. The larva feeds first internally, then externally on 3 to 4 seeds, and after completion of its development spins a white cocoon within the pod. Emergence occurs through a circular hole in the pod wall (Fig. 8). Infestation rate in the typical locality is rather high: out of 88 pods collected in 2003, at least 10 were infested by the bruchid (other pods may have been infested but did not yield any adult).

REMARK

CAILLOL (1954) mentions *A. depressus* as a host plant of *B. nanus* in Provence (south-eastern France). Externally similar to *B. bernardi*, *B. nanus* is a different species which apparently feeds exclusively on *Medicago* seeds (tribe Trifolieae).

PHYLOGENETIC ANALYSIS

Parsimony analysis of the partial 12S rRNA gene yielded a single tree of 124 steps (CI = 0.838; RI = 0.764; RCI = 0.641) shown in Fig. 9. Uncorrected pairwise distances among the different taxa (Table 3) ranged from 0.0075 to 0.1203.

Our topology (Fig. 9) clarifies the position of *B. bernardi* in relation to three other European *Bruchidius* species associated with Galegeae. A well supported clade (bootstrap support value of 100%) includes three of the four species feeding on *Astragalus* (*B. bernardi*, *B. caninus* and *B. marginalis*). These species show a low level of sequence divergence (below 2%) and have probably diverged recently. Interestingly, *B. poecilus* is phylogenetically more related to the species feeding on Trifolieae.

The results of the analysis of the 12s rRNA gene are in greater part supported by morphological evidence. In particular, the shape of the aedeagus is quite similar in *bernardi*, *caninus* and *marginalis*; one notable difference between them is the presence of a pointed and hairy expansion on the dorsal valve in *B. caninus*, which is absent in the other three species. Contrary to what might have been

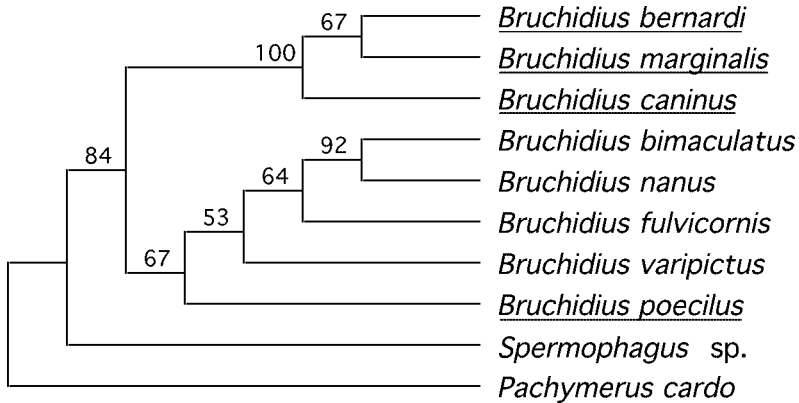
expected, *poecilus* and *bernardi* show a similarly enlarged 4th male antennal segment, and the elytral pattern, with apical and lateral black spots on a whitish background, exists in both *poecilus* and *marginalis*. But it exists also in members of the *varius* group. *B. caninus* is an intermediate case, with specimens feeding in *A. hamosus* seeds showing a partial lateral black spot while those from *A. massiliensis* are devoid of the black spot. The most striking difference between *poecilus* and the other three species from Galegeae lies however in the shape of parameres: they are thin and their apex is highly modified in *poecilus*, much in the same way as in the *Bruchidius varius* and *Bruchidius murinus* groups (BOROWIEC 1988), whereas in *bernardi*, *caninus* and *marginalis* lateral lobes are short, stout, with an enlarged but unmodified apex, as expected in members of the *astragali*-group (see Fig. 3).

Tab. 3: Uncorrected pairwise distance matrix

Species	1	2	3	4	5	6	7	8	9	10
1. <i>B. bernardi</i>	–									
2. <i>B. bimaculatus</i>	0.0729	–								
3. <i>B. caninus</i>	0.0175	0.0679	–							
4. <i>B. fulvicornis</i>	0.0677	0.0175	0.0652	–						
5. <i>B. marginalis</i>	0.0075	0.0704	0.0100	0.0652	–					
6. <i>B. nanus</i>	0.0752	0.0075	0.0702	0.0200	0.0727	–				
7. <i>B. poecilus</i>	0.0651	0.0404	0.0594	0.0374	0.0571	0.0404	–			
8. <i>B. varipictus</i>	0.0707	0.0309	0.0678	0.0281	0.0679	0.0364	0.0491	–		
9. <i>P. cardo</i>	0.1155	0.1003	0.1128	0.1100	0.1180	0.1051	0.1065	0.1081	–	
10. <i>S. sp.</i>	0.0930	0.0957	0.0955	0.0879	0.0880	0.0955	0.0891	0.0925	0.1203	–

The new species clearly belongs to the same group as *B. marginalis* and *B. caninus*, and may therefore be assigned to the *astragali*-group. It may be noted that the latter two species have at least one host in common, namely *A. hamosus*, a fact which may be linked with their close phylogenetic relationship.

The position of *B. poecilus* must be considered with caution at present. Our results show that *B. poecilus*, an *Astragalus* feeder, seems more closely related to species feeding in Trifolieae seeds than with species feeding in *Astragalus*. Our knowledge of host-plant relationships in the groups of species under study is however far too scanty to allow a complete clarification of its position. Numerous species of *Astragalus*, *Medicago* and *Trifolium* have never been sampled for seed-beetle infestation in Southern Europe, Northern Africa and Western Asia, so that major findings may be expected when more biological data become available. Further research should reveal whether the species groups as currently defined are a morphologically, genetically and biologically satisfactory description of reality.



9. Most parsimonious tree from the unweighted parsimony analysis of the partial 12s rRNA gene (numbers above branches are bootstrap support values). *Astragalus*-feeding species names are underlined

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