Phylogenetic relationships of a new species of seed-beetle infesting *Cercis siliquastrum* L. in China and in Europe (Coleoptera: Chrysomelidae: Bruchinae: Bruchini)

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Abstract. *Bruchidius siliquastri* **n. sp.** is described. This seed–beetle develops in pods of *Cercis siliquastrum*, a widely grown ornamental tree. Several recent reports reveal that this species is well established in Southern France. The presence of this species is also reported from Hungary. The relationships of this new species are investigated using both morphological data and molecular phylogenetic analyses. Our results indicate that this species is likely related to a mostly Paleotropical group, which includes members of genera *Bruchidius* and *Conicobruchus*. Hypotheses on the geographic origin of this new species are also discussed.

Résumé. Affinités phylogénétiques d'une nouvelle espèce de bruche ravageuse de *Cercis siliquastrum* L. en Chine et en Europe (Coleoptera : Chrysomelidae : Bruchinae : Bruchini). *Bruchidius siliquastri* **n. sp.** est décrite. Cette bruche se développe dans les gousses de *Cercis siliquastrum*, un arbre ornemental qui est fréquemment planté à travers le monde. Des observations récentes révèlent que cette espèce est bien installée dans le sud de la France. La présence de cette espèce en Hongrie est également rapportée. Les liens de parenté de cette nouvelle espèce sont examinés à l'aide de données morphologiques et d'analyses phylogénétiques basées sur des caractères moléculaires. Nos résultats indiquent que cette espèce est vraisemblablement apparentée à un groupe majoritairement paléotropical, qui inclut des membres des genres *Bruchidius* et *Conicobruchus*. Des hypothèses concernant l'origine géographique de cette nouvelle espèce sont également discutées.

Keywords: *Bruchidius siliquastrum*, *Conicobruchus,* Judas tree, France, Hungary.

The genus *Cercis* belongs to the family Leguminosae, subfamily Caesalpinioideae, tribe Cercideae; its various species are commonly known as "redbuds". Up to ten species are currently recognized (Isely 1975; Tralau 1981; Salatino *et al*. 2000; Davis *et al*. 2002). Two of them (*Ce. canadensis* L. 1753 and *Ce. occidentalis* Torr. ex A. Gray 1850) are native to North America, while six species originate from China, one species (*Ce. griffithii* Boiss. 1873) originate from Central Asia, and *Ce. siliquastrum* L. 1753 from Central Asia to Europe (ILDIS, 2006). *Cercis siliquastrum* (Mediterranean redbud, Judas tree, "*arbre de Judée*") is grown in all temperate regions of the world for its profusion of pink to magenta flowers. Chinese species are less commonly found in gardens, but are quite frequently planted in parks and arboretums.

Most Bruchinae recorded from Cercideae feed on seeds of various species of *Bauhinia*; they belong to four different genera: *Caryedon, Caryedes, Gibbobruchus* and *Pseudopachymerus*. A single species, *Gibbobruchus mimus* (Say 1831), is known to predate on the two Nearctic species of *Cercis* (Cushman 1911; Zacher 1952; Whitehead & Kingsolver 1975; Center & Johnson 1976, Johnson 1977; Hetz & Johnson 1988). The record of *Callosobruchus maculatus* (Fabricius 1775) on *Cercis canadensis* by Zacher (1952) is very doubtful and has not been confirmed. No seed-beetle has been so far recorded on any Old World species of *Cercis*.

In 2003, three specimens of seed-beetle emerged from a small sample of *Ce. siliquastrum* pods collected in Montpellier (Southern France, Hérault). These specimens proved to be new to Science and without known relative in Europe. They were found to be conspecific with specimens bred in China from an unidentified species of *Cercis* (K.-W. Anton, pers. comm.). Three years later, the species was collected in the Rhône valley (Valence, and as far north as Villefranche-sur-Saône). This seed-beetle was also reported from Southern France in the following localities: Cadalen (Tarn) (A. Sergent, pers. comm.), Gémenos (Bouches-du-Rhône), La Roquebrussanne (Var) (P. Ponel, pers.

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comm.) and Nice (Alpes-Maritimes) (M. Clément, pers. comm.). It was also recently reported from Hungary (O. Merkl, pers. comm.)

In this study, the new species is described and its relationships with other bruchines are investigated using both morphological data and molecular phylogenetic analyses.

Material and Methods

Samples of recently matured pods of *Cercis siliquastrum* were first collected in Montpellier in June 2003. Additional material was collected from the same location during the summer of 2004, from Valence (Drôme) in 2005 and from Villefranche-sur-Saône (Rhône) in early 2006. Pods were kept in aerated plastic vials until emergence of adults.

Morphological analysis

Male and female genitalia were mounted on microscope slides in water-soluble DMHF (dimethyl hydantoin formaldehyde). Figures of genitalia were drawn from microphotographs of slide preparations. Male and female habitus were captured using a Canon EOS 350D digital single lens camera with a MPE 65 mm MACRO lens. Body length was measured from apex of pronotum to apex of elytra. The terminology of genital parts follows Kingsolver (1970).

Molecular phylogenetic analyses

DNA was extracted from an ethanol preserved specimen using the Qiagen DNeasy tissue kit (Qiagen, GmbH, Germany). Partial sequences from three mitochondrial genes (12S rRNA, cytochrome *b* and cytochrome *c* oxidase subunit I) were obtained using standard protocol described elsewhere (Kergoat *et al.* 2004). In order to minimize the computational time of the phylogenetic analyses, a two-steps strategy was followed.

First, we have conducted preliminary analyses on a large data set of 152 species to identify the closest relatives of the new species. Genbank sequences (as well as unpublished data from G.J. Kergoat) from four genes (12S rRNA, cytochrome *b*, cytochrome *c* oxidase subunit I and 28S-D2 rDNA) were concatenated and aligned using ClustalX (Thompson *et al.* 1997) with default settings. MrBayes 3.12 (Huelsenbeck & Ronquist 2001) was used to perform partitioned Bayesian analyses (one partition per gene; two distinct runs of 10 million generations; burnin period of 100,000 generations). For each gene, the best-fit substitution model was determined using Modeltest (Posada & Crandall 1998). In all analyses, *Pachymerus cardo* (Fahraeus 1839) (Chrysomelidae: Bruchinae: Pachymerini) and *Gibbobruchus* sp. (Chrysomelidae: Bruchinae: Bruchini) were used as outgroups, following previous studies (e.g. Kergoat *et al.* 2005).

More thorough analyses were subsequently performed on the data set determined by preliminary analyses, using two inference methods (Bayesian inference and parsimony). Bayesian inference analyses were carried out with the settings used beforehand. For parsimony, the program PAUP* 4.0b10 (Swofford 2002) was used to perform unweighted analyses with the following options: heuristic search with 1,000 random-addition replicates; *Tree-bisection-reconnection* (TBR) algorithm; *Maxtrees* set to 1,000; *Random addition sequences*. Since a preliminary incongruence length difference test (ILD; Farris *et al.* 1994) was not significant, the whole data set was analyzed simultaneously. In all parsimony analyses, gaps were considered as a fifth character following Giribet & Wheeler (1999). The robustness of trees was assessed by non-parametric bootstrap procedures (1,000 replicates; Felsenstein 1985) for parsimony analyses and by clade posterior probabilities (CPP) for Bayesian inference analyses (Huelsenbeck *et al.* 2002).

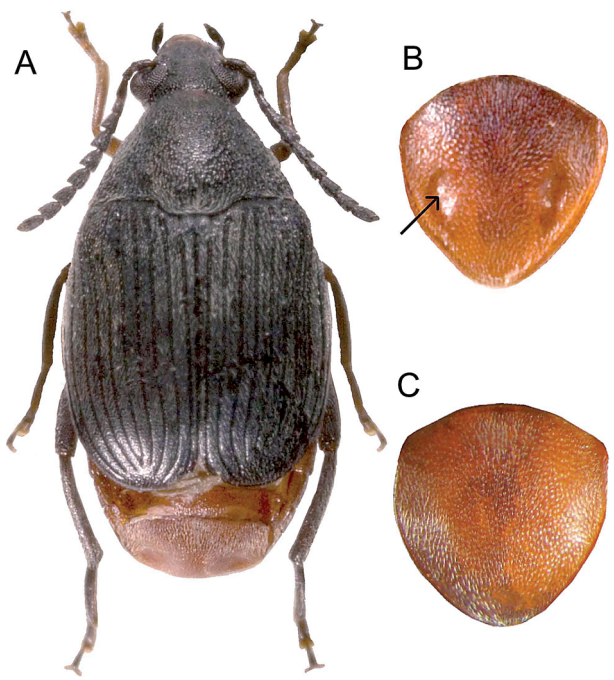

Figure 1
Bruchidius siliquastri **n. sp. A,** habitus of female; **B,** female pygidium, showing foveae; **C,** male pygidium.

Results

Description of the new species

Bruchidius siliquastri Delobel, n. sp.

Type material. Holotype. ♂ «France, Hérault, Montpellier, rue Las Cazes [43°37'03"N 3°51'01"E], 30.VII.2004 ex *Ce. siliquastrum*, C. & P. Delobel coll. CP97 » « *Bruchidius siliquastri* n. sp., A. Delobel des. 1997 » « HOLOTYPE » [red]. Paratypes: 16 males, 21 females, same data and collectors as holotype; 13 males, 19 females, same data, but 24.VII.2004, ENSA Bat. 28, CP92; 1 male, 2 females, same data, but June 2003, P. Delobel coll., Fr 33; all specimens in the collections of MNHN. **Additional material.** 1 male, 1 female, France, Drôme, SEFRA [43°37'N 3°51'E], 8.VIII.2005, ex *Ce. siliquastrum,* P. Delobel coll., CP051558.

Description. Length (pronotum-pygidium): 2.8–3.7 mm.

Body (fig. 1) short ovate, integument, including legs and antennae, black, abdomen yellowish–red, except ventrite 1 partly black basally. Often extreme apex of femora and ventral

part of hind tarsi very slightly reddish; 4 anterior (specially fore) legs sometimes not black, but rather of a dark reddish brown. Vestiture made of thin and short setae, rather dense, though not completely covering integument, recumbent, except on basal part of ventrite 1; dorsally composed of whitish setae, less dense on sides and apex of elytra, denser on disc and scutellum; on pronotum, setae longer on disc and basal lobe, becoming shorter and less dense on sides; pygidium almost uniformly covered with short and thin setae; setae moderately long on ventral side.

Male. Head short; eyes bulging, maximum head width 1.43 times width behind eyes; eyes separated by 0.35 times head width including eyes; distance between posterior rim of eyes and apex of clypeus / distance between eyes = 1.76; eye cleft to about half of its length, width at bottom of sinus composed of 7-8 ommatidia; maximum width of postocular lobes equal to 1/3"' eye width at sinus; carina on frons absent, no interocular tubercule. Antenna of moderate length, slightly surpassing elytral base; antennal segments 2-3 subcylindrical, almost equal in length, segments 5-11 widened at apex, but always longer than wide, 11 oval (L/W = 2.1). Length of antennomeres: 1.4 : 1 : 1.4: IA: 1.5 : 1.6: 1.6 : 1.7: 1.7 : 13: 2.6. Pronotum strongly convex, with greatest width at base (W/L = 1.57),subtrapezoidal, its sides convex at about 2/3 of its length from base; without oblique impression on sides of basal lobe. Its disc with strong, well separated punctures, becoming stronger and slightly coriaceous laterally; punctuation suddenly interrupted on posterior half of sides, where integument is alutaceous, with a slight carina-like separation between the two areas. Elytra short, 1.04 times longer than combined width, their sides strongly convex, maximum width at middle; disc flattened; base of interstriae 2-4 margined, with a very small tooth at base of stria 3. Striae on disc well defined; interstriae flat, strongly alutaceous, with irregular lines of larger punctures. Hind femora incrassate, at their widest 1.8 times wider than mid femora; mesoventral margin carinate, with small preapical denticle, immediately followed by a much shorter one (visible only at higher magnification); hind tibiae widened apically, with dorsomesal and ventral carinae complete and strong, lateral not reaching base; apex of tibia with mucro shorter than width of tarsomere 1 at base; lateral denticle as long as mucro, and dorsal denticles 1/2 of lateral denticle. First tarsomere ventrally without apical denticle.

Abdomen with ventrite 5 emarginate, medially as long as sternite 4; ventrite 1 basally with a large patch of short erect setae reaching beyond middle of ventrite; these setae slightly scaly, 2–3 times shorter and much denser than those on surrounding integument (fig. 2). Pygidium subcircular (W/L = 1.01), with apex turned under (see fig. 1).

Genitalia (fig. 3): Median lobe of moderate length (maximum width excluding basal hood / total length = 0.27); ventral valve subtriangular, moderately sclerotized, bearing numerous sensillae and on each side a row of 10 to 13 short setae; valve flanked by a pair of large hinge sclerites; internal sac with two or three large pointed denticles. Basal strut without keel. Lateral lobes cleft to 0.6 their length; apex of parameres with numerous long setae.

Female. Similar to male, but last abdomina1 ventrite not emarginate, longer than 4th ventrite, pygidium slightly narrower than in male (L/W = 1.05), bearing a pair of elongated foveae (see fig. 1); foveae 0.3 to 0.4 mm long, 0.1 wide, moderately deep, unmargined, their bottom without setae, slightly alutaceous. Genitalia: spiculum gastrale as in fig. 4; spermathecal body narrow and strongly curved, with an elongated apical diverticulum; opening of spermathecal gland

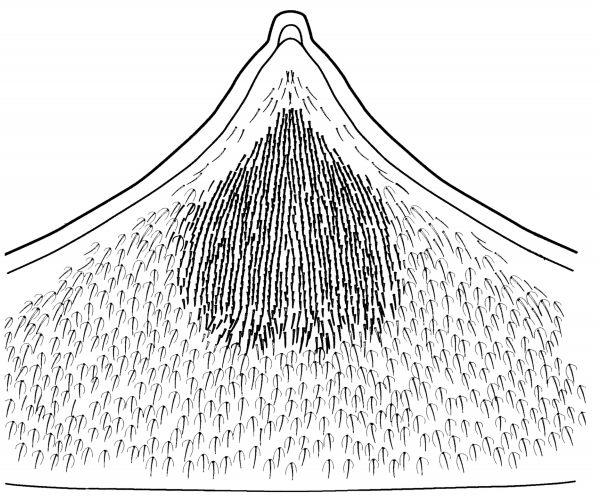

Figure 2
Bruchidius siliquastri **n. sp.** Detail of male ventrite 1, showing the large setiferous patch.

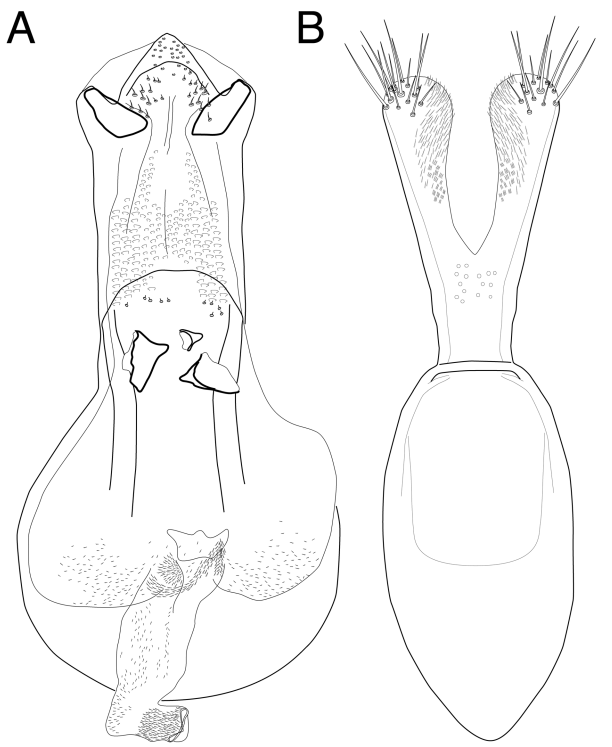

Figure 3
Bruchidius siliquastri **n. sp.** Male genitalia, ventral view. **A,** median lobe; **B,** tegmen.

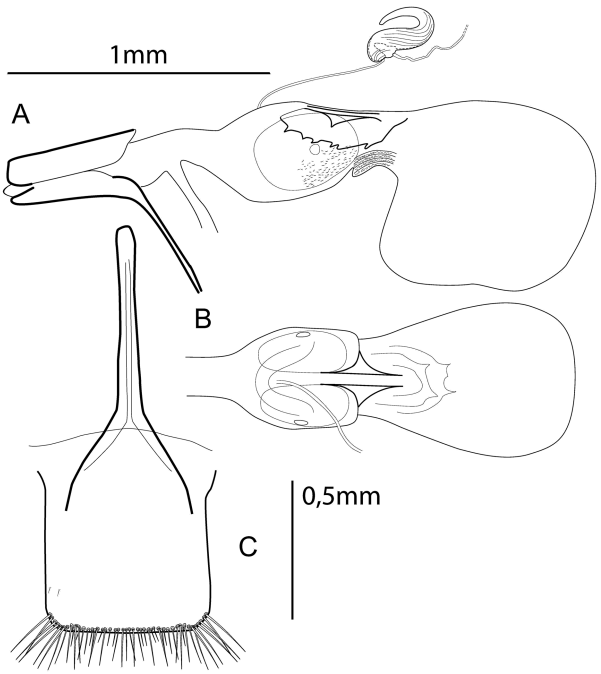

Figure 4
Bruchidius siliquastri **n. sp.** Female genitalia. **A,** lateral view; **B,** dorsal view; **C,** speculum gastrale.

duct basal, contiguous with spermathecal duct opening; vagina with a large sclerite on dorsal side, a little behind spermathecal duct opening (fig. 4). Bursa copulatrix without spines or needles.

Host plants. All examined material was reared from mature seeds of *Cercis siliquastrum.*

Etymology. The name refers to the name of the host-plant, *Cercis siliquastrum*.

Distribution. China, France and Hungary. Probably present in several other European countries.

Molecular phylogenetic analyses

In all preliminary analyses, *B. siliquastri* was found clustered within a clade constituted by species belonging to genera *Bruchidius* Schilsky 1905, *Conicobruchus* Decelle 1951 and *Megabruchidius* Borowiec 1984. Based on this result, a subset of 21 species (including the two outgroup species) was defined (see tab. 1) and analyzed following the methods described above.

A mean sequence divergence (K2P distance) of 16.68 ± 0.72% is found between *B. siliquastri* and the other 19 ingroup species (the closest species being *Conicobruchus strangulatus* (Fahraeus 1839), with 15.51% of divergence). This value is consistent with the mean sequence divergence of 15.53 ± 3.70% that was found when considering the whole subset of 21 species. Bayesian inference and parsimony analyses yield topologies that are quite distinct (fig. 5). Under

Table 1. Taxon sampling.

Species	Taxonomic group (if any)	Locality
Bruchidius antennatus (Wollaston 1864)	***biguttatus***	Tenerife, Canary Islands
Bruchidius biguttatus (Olivier 1795)	***biguttatus***	Gard, France
Bruchidius chloroticus (Dalm. 1833)		Fatick, Sénégal
Bruchidius cisti (Fabricius 1775)	***cisti***	Umbria, Italy
Bruchidius holosericeus (Schönherr 1832)	***holosericeus***	Kfar Selouane, Lebanon
Bruchidius lichenicola (Wollaston 1854)	***lichenicola***	La Palma, Canary Islands
Bruchidius lineatopygus (Pic 1924)	***lineatopygus***	Louga, Sénégal
Bruchidius lutescens (Blanchard 1844)	***cisti***	Fokida, Greece
Bruchidius nodieri (Pic 1943)		Louga, Sénégal
Bruchidius pauper (Boheman 1829)	***pauper***	Haute Corse, France
Bruchidius poupillieri (Allard 1868)	***cisti***	Basilicata, Italy
Bruchidius siliquastri **n. sp.**		Hérault, France
Bruchidius unicolor (Olivier 1795)	***cisti***	Vaucluse, France
Conicobruchus albopubens (Pic 1931)		Cabo Verde, Sénégal
Conicobruchus indicus (Pic 1909)		India
Conicobruchus strangulatus (Fahraeus 1839)		Cabo Verde, Sénégal
Gibbobruchus sp.		Guyane française
Megabruchidius dorsalis (Fahraeus 1839)		Japan
Megabruchidius tonkineus (Pic 1904)		Hô Chi Minh-Ville, Vietnam
Megabruchidius sophorae Tuda & Morimoto 2004		Tsukuba, Japan
Pachymerus cardo (Fahraeus 1839)		Guyane française

parsimony, two most-parsimonious trees (3075 steps; CI = 0.459; RI = 0.357) are found, which only differ by the position of *B. lichenicola* (Wollaston 1854). The latter species is found either as the sister species of *Conicobruchus indicus* (Pic 1909) or in a more basal position (see fig. 5). Interestingly, both methods recover three clades that are similar in composition (entitled A, B and C; see fig. 5). Clade A groups together the three species of *Megabruchidius*, with a high support (CPP and bootstrap of 100%). Clade B is constituted by African and Asian species (with the exception of *B. lichenicola*, which is endemic to the Canary Islands) belonging to the genera *Bruchidius* and *Conicobruchus*. In this clade *Bruchidius siliquastri* is found either in a basal position, under Bayesian inference (CPP of 75%), or clustered with *Co. strangulatus*, under parsimony (bootstrap of 29%). Finally, clade C gathers the European species belonging to the *Bruchidius biguttatus-cisti-holosericeus-pauper* groups (*sensu* Borowiec 1988).

Discussion

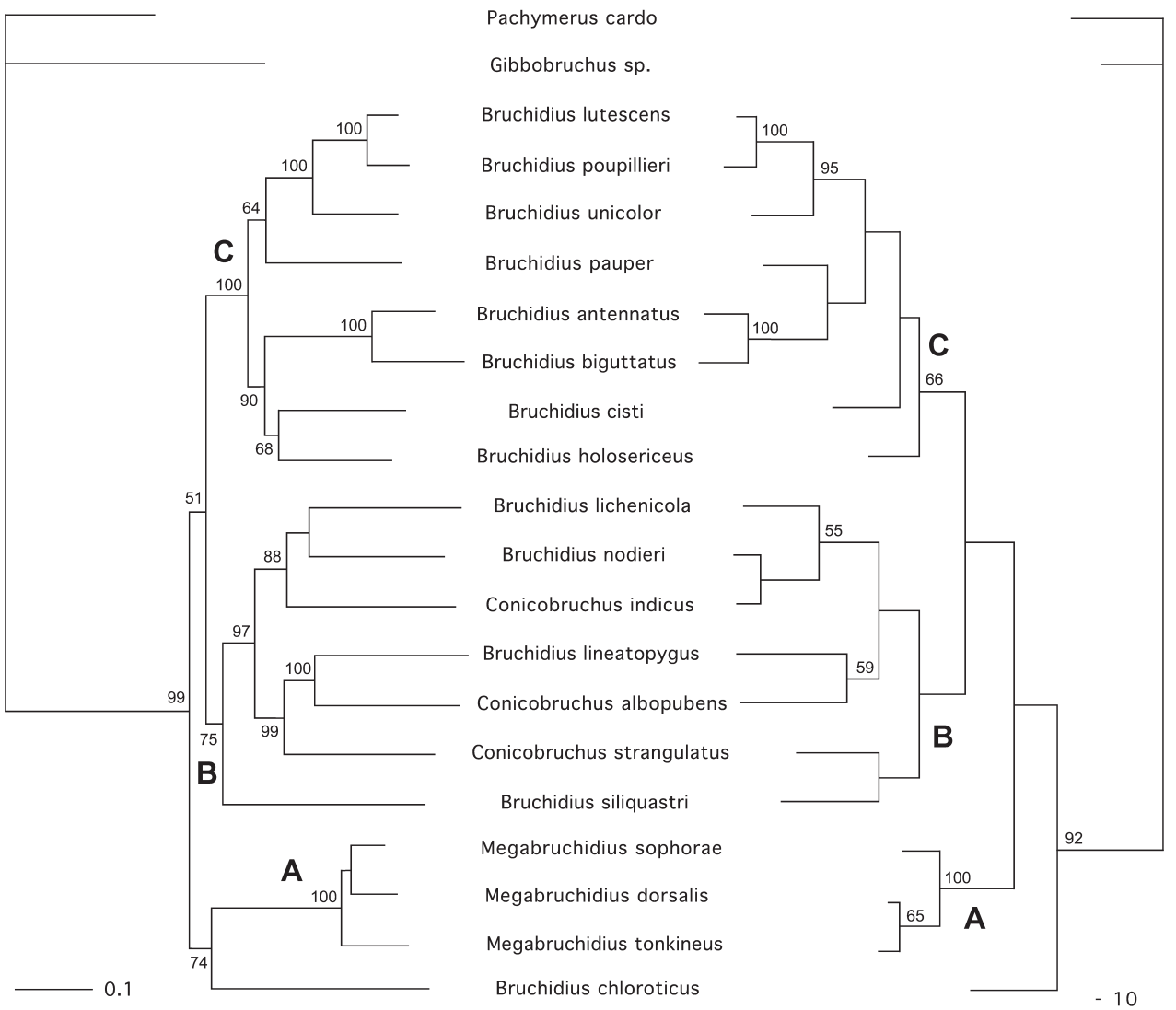

Figure 5
Phylogenetic relationships of the sampled bruchine species. The tree of the left corresponds to the result of the Bayesian inference analyses with numbers at nodes indicating CPP values (only values >50% are figured). The tree on the right corresponds to one of the two most-parsimonious trees from the parsimony analysis of the combined data set with numbers at nodes indicating bootstrap values (only values >50% are figured). The three clades that are recovered by both analyses are indicated by capital letters (A, B and C).

Male morphology is characterized by the presence of a large setiferous patch on the first ventrite. Abdominal setiferous areas were described in a number of members of the subtribe Acanthoscelidina: *Acanthoscelides lobatus* (Fall 1910) (Johnson 1970), *A. quadridentatus* (Schaeffer 1907), *Bruchidius aurivillii* (Blanc 1889), *B. uberatus* (Fahraeus 1839), *Megabruchidius tonkineus* (Pic 1904) (Faustini & Halstead 1982), *Scutobruchus ceratiobruchus* (Philippi 1859) (Kingsolver 1968), and is present in a large number of tribes, including the basal tribe Pachymerini. The presence of large hinge sclerites in the median lobe of the new species is a feature that is rather common in various African and Asian species presently known as *Bruchidius* or *Conicobruchus* spp., particularly in *B. bimaculatopygus, B. confusus* and *B. lineatopygus*. The particular conical sclerites of the internal sac are also of a rather uncommon type. Similar sclerites are found in *Bruchidius danilevskyi* Anton 1999, *B. sapamoroensis* Kingsolver 1984, *B. savitskyi* Anton 1999 and *B. tuberculicauda* Lukjanovitch & Ter-Minassian 1957. But the latter four species share a ventral valve that is definitely more pointed than in the present species. Female morphology is characterized by the presence of a pair of longitudinal depressions located on the sides of the pygidium. Pygidial depressions were so far known only in the *B. centromaculatus* group (Faustini & Halstead 1982; Anton & Delobel 2003); foveae in *B. siliquastri* are however quite distinctive because they are not limited by any sort of carina.

Molecular analyses indicate a close relationship between *B. siliquastri* and an assemblage of species belonging to the genera *Bruchidius* and *Conicobruchus* (clade B in both trees). This grouping is also suggested by the fact that a member of this group, *Co. strangulatus*, exhibits the lowest level of sequence divergence with *B. siliquastri*. The level of support for the clade B is not high, as indicated by a moderately high CPP value (75%) and by low bootstrap values (below 50%). Having said that, the results of molecular analyses are consistent with the morphological evidence that brings together *B. siliquastri* with the species that possess similar large hinge sclerites in the median lobe. Interestingly the latter character is shared by all species belonging to clade B, with the exception of *B. lichenicola*.

Though our initial sampling of 152 species was quite dense (for instance, *B. aurivillii* and members of *B. centromaculatus* group with pygidial depressions were included), we only partly manage to get some of the key-species that possess one of the characteristic features of *B. siliquastri* (i.e., particular sclerites of the internal sac, pygidial depressions or setiferous areas). In the absence of a denser sampling, the hypothesis of a close relationship between *B. siliquastri* and the species related to the other members of clade B thus appears as the most likely. In addition, our analyses reveal the paraphyly of the genus *Conicobruchus*, and the paraphyly of the group *cisti*. These preliminary results highlight the need of pursuing studies in the taxonomy and systematics of Old-World seed-beetles in order to clarify their phylogenetic relationships. They justify our present conservative decision not to create a new genus, and to ascribe the new species to the genus *Bruchidius*.

The fact that this species was also reported from unidentified species of *Cercis* in China, and its relatedness with non-European bruchines allows us to discuss of the likely alien status of this species in Europe. Given the rather conspicuous appearance of *B. siliquastri* (at least for a seed-beetle) and the wide native area of distribution of *Ce. siliquastrum* (from Europe and Central Asia) it seems difficult to explain why this seed-beetle was not discovered earlier. A recent shift of *B. siliquastri* from Oriental species of *Cercis* toward *Ce. siliquastrum* thus appears as the most likely explanation. We can postulate that this species is now present in many other countries (and perhaps in the New World too), due to the popularity of the European redbud as an ornamental tree. To conclude, we suggest surveying the possible development of this seed-beetle species on other commonly grown *Cercis* species, and especially on the two New World species that appear to be closely related with *Ce. siliquastrum* according to recent molecular analyses (Davis *et al.* 2002).

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