CRESSA CRETICA L. (CONVOLVULACEAE), HOST-PLANT OF THE WEEVIL **SHARPIA RUBIDA** (ROSENHAUER, 1856) (COLEOPTERA, CURCULIONIDAE, ERIRRHININAE)

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Abstract
In the course of a study on **Cressa cretica**, an endangered plant living in transient salt marshes, we detected a parasitism involving the rare weevil **Sharpia rubida**. The consequences for the reproduction of the plant may be dramatic, since up to about 90% of the flower buds are destroyed. Oviposition and larval development of **Sharpia rubida** occur in the flower buds between the end of July and the end of August. The fourth-instar larvae pupate in the soil. Adults emerge in September and overwinter in the surrounding vegetation.

Key-words
Parasitism, host-plant, salt marshes, endangered species, **Cressa**, **Sharpia**, larval development, chorology

Résumé. **Cressa cretica** L. (**Convolvulaceae**), plante-hôte du charançon **Sharpia rubida** (Rosenhauer, 1856) (***Coleoptera***, ***Curculionidae***, ***Erirrhininae***).
Une étude portant sur **Cressa cretica**, une plante menacée des mares temporaires halophiles, a permis de déceler un parasitisme dû à un rare charançon : **Sharpia rubida**. L'impact sur la reproduction est très important, puisque jusqu'à près de 90% des boutons floraux sont détruits. La ponte et le développement larvaire de

Sharpia rubida se déroulent dans les boutons de fin juillet à fin août. Les larves de quatrième stade se nymphosent en terre. Les adultes éclosent en septembre et hibernent dans la végétation environnante.

Mots clés
Parasitisme, plante-hôte, mares halophiles, espèce menacée, **Cressa**, **Sharpia**, développement larvaire, chorologie

Introduction

Cressa cretica L., a Convolvulaceae with a thermo-cosmopolitan distribution (PIGNATTI, 1982), is found is Southern Europe, Asia and Africa (AUSTIN, 2000). The plant is rare in France, it is protected in the Provence-Alpes-Côte d'Azur Region, and listed as a priority species in the French National Red Book (LAVAGNE, 1995). **Cressa cretica** is a clonal, perennial and halophyte species. It develops long underground rhizomes reaching up to 1 meter in depth. In the Camargue (delta of the Rhône river region), the plant is found during the dry phase of transient marshes, where it develops over the summer period and disappears when the water returns. 30 years ago, it was known from only two locations respectively on and near the Tour du Valat estate (MOLINIER & TALLON, 1974). One of these stations was destroyed in the 70's by a change in water management. However, the plant is now found in large populations around 16 marshes on the Tour du Valat estate. An additional station was recently found (2006) on the neighbouring Réserve Nationale de Camargue (Oliver A. & Coulet E., pers. com.). The very low seed production observed in these populations contrasted with the abundant flower bloom and with the current trend of spreading populations. In this context a survey of the Camargue populations of **Cressa cretica** was initiated in 1998 to understand better the low seed yield.

The main and unexpected result was that about 90% of the flower buds are found destroyed. The dissection of some of these flower buds showed that they were infested with weevil larvae. Follow-up surveys in the field in 1999, resulted in the discovery, near and on the plants, of adult insects belonging to the species **Sharpia rubida** (Rosenhauer, 1856), a most uncommon weevil in France (HOFFMANN, 1958).

The genus **Sharpia**, described by Tournier, 1874 belongs to the tribe **Smicronychini** and is akin to the genus **Smicronyx** Schönherr, 1843. Both genera are characterized by the eye contiguity under the head but **Sharpia** differs by the second article of the funicle elongated, the third tarsomere neither bilobate nor fluffy on its underside, and the onychium much longer.

The genus is distributed in the southern parts of the Palearctic region,

including Siberia. It contains about 10 species, with the highest species diversity in the eastern Mediterranean region. The species **Sharpia rubida** is found across the Mediterranean basin and is the only species of the genus in the western Mediterranean region. We found it in Northern Senegal in 1999. It has however always been considered a rare species: « *Sharpia non è mai stato studiato in maniera adeguata anche per la difficoltà di riunire materiali abbondanti di raccolte non occasionali.* » (OSELLA and DI MARCO, 1996; see also ALONSO-ZARAZAGA, SAN VICENTE and COELLO, 2009 for a review on the species distribution). In France, over the past 100 years, only a handful of specimens were collected – to list a few citations from the literature: Albaron (Bouches-du-Rhône), Hyères, Le Luc (Var) (CAILLOL, 1954; HUSTACHE, 1930). It was also recorded from salt marshes in North Africa on ***Suaeda*** plants (Caillol, 1954) under the names *biskrensis* Desbrochers, 1875 and *gracilenta* Fairmaire, 1877, later synonimized with **Sharpia rubida** by BEDEL (1888).

HOFFMANN (1958) claims to have obtained **Sharpia rubida** adults from breeding larvae found at the base of ***Atractylis humilis*** L. (Asteraceae) from La-Roquette-sur-Siagne (France, Alpes-Maritimes). But THÉROND (1976) questions this record, arguing that this plant is absent from Camargue, while **Sharpia rubida** was collected in this region multiple times.

We have undertaken to better understand the life cycle of **Sharpia rubida** and its relationship to its host-plant, through direct field observation of adults, and the survey of larvae in flower buds and in the soil.

Methods

Sharpia rubida on Cressa cretica plants

In the course of a weekly survey during 2 consecutive summers (August 14th – September 9th, 1998 and July 7th – August 27th, 1999) of **Cressa cretica** populations on 7 pools on the Tour du Valat Nature preserve (Figure 1D), ramets of Cressa were sampled and kept frozen (-18°C) for *a posteriori* scoring of the larval development. A total number of 7,515 flower buds were checked (2,605 in 1998 and 4,910 in 1999). Flower buds were dissected under a stereomicroscope and the number of attacked buds was counted. We recorded as « attacked » any bud that contained a larva, was wounded at its base, or had obviously been emptied by a now-gone larva.

To measure the density of larvae when they leave the flower buds and the subsequent time needed to reach the adult stage, larvae were captured with traps and raised in the laboratory. On one site, Tamarguiron, during a pilot study, 21 traps were randomly distributed (21-28 July 1999) within the populations of **Cressa cretica**. On Baisse des Tirasses site, 31 traps were randomly distributed in 2000 (during 224 h for intermittent catch cumulated captures between July 21st and August 8th). Traps were aluminium boxes (125 cm²) fitted at the soil

level and filled with 2 cm of fine sand. In 2000, among the 225 larvae caught, 32 were then raised in the laboratory in cells filled with sand until they reach the imago stage.

Density of larvae in the soil

In order to estimate the density of larvae in the soil, samples of soils were taken at 2 sites, Cerisières sud and Baisse des Tirasses: On August 10th and October 14th 1999, we sampled the soil at the site Cerisière sud (digging with a shovel) at a depth of 5-20 cm across a surface of 30 x 40 cm. The sample was split into 5 cm deep layers. On August 26th 1999, on the same site, we made another 10 soil samplings with a drill, at a depth of 45-60 cm, and split the resulting cores into 10 cm deep subsamples. Finally, on August 8th and 9th 2000, at Baisse des Tirasses, 31 cores of soil (surface: 4.5 x 4.5 cm; depth: 0-20 cm, split into 5 cm layers) were taken at random.

To extract the larvae the cores were carefully washed on sieves with water, and, when necessary, processed onto a Berlese funnel extractor.

Observations of imagos

During this survey, adult **Sharpia rubida** were occasionally collected on the plants of **Cressa** or near their base. In addition, evidence of the presence of **Sharpia rubida** was found during an inventory of the known populations of **Cressa cretica** along the French Mediterranean coast (18 pools visited from August to October 2000, see Results).

Larval development

The larvae obtained in 1999 from the flower buds, from the traps and from sediment cores were measured to understand the phenology of the larval development better. Staging of larvae was based on a measure of the width between mandibular articulations on 291 larvae collected at different time over the summer 1999 on the plant or in the soil. The distribution of the width between the mandibular articulations (n = 291) resulted in 4 discrete, non-overlapping groups of animals (data not shown). This distribution reflects the four larval stages normally found in Curculionidae (MAY, 1994).

Larval description was based on 10 fourth-instar specimens stored in 70% ethanol. It was carried out under low magnification (Leica MZ6 stereoscope) for the general characters. The drawings of the head and spiracles (Figure 2A) were obtained from microscopic preparations (MAY, 1994) observed and photographed using a Zeiss Axiophot compound microscope equipped with a 20x dry lens.

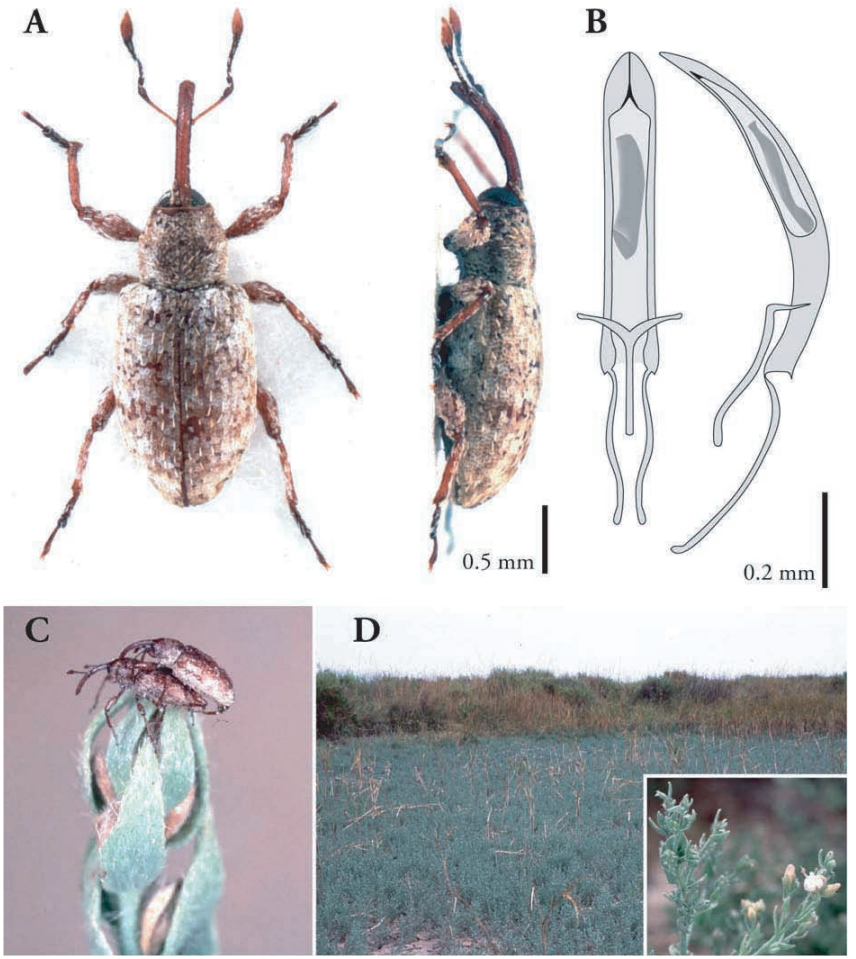

Figure 1. The imago of **Sharpia rubida**. A, an adult male in dorsal and lateral views; B, drawings of the male aedeagus in dorsal and lateral view; note the presence of an heavily sclerotised internal sac; C, a couple of **Sharpia rubida** adults mating on a stem of **Cressa cretica**; D, the typical habitat of **Cressa cretica** and **Sharpia rubida**, inset: a close-up view of a flowering **Cressa cretica**.

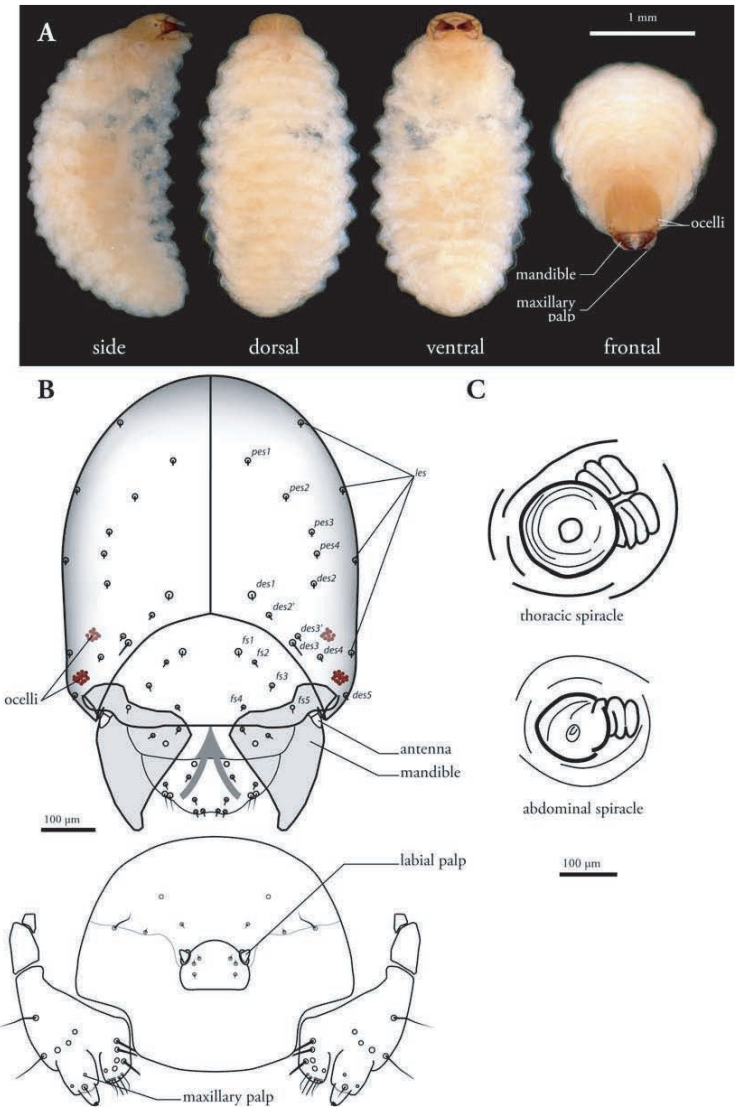

Figure 2. External morphology of the fourth-instar larva of **Sharpia rubida**. A, photographs of a larva from various angles (dorsal is to the left on first view, anterior is up in first three views); B, schematic, annotated frontal view of the cephalic capsule (top), maxilla and labium (bottom); C, schematics of the thoracic and abdominal spiracles. Abbreviations: pes: posterior epicranial setae; les: lateral epicranial setae; des1-5: dorsal epicranial setae 1 to 5; fs1-5: frontal setae 1 to 5.

Imaging

An adult (Figure 1A) and a larva (Figure 2A) were imaged on a Leica Z6 Apo microscope. A stack of images taken at different focal planes was projected using the StackFocusser plug-in in the ImageJ software (http://rsbweb.nih.gov/ij/).

Results

Survey of the imagos

During the weekly field survey of the 7 pools of La Tour du Valat, adult specimens of **Sharpia rubida** were observed from July 26th to August 4th 1999. They were crawling on the ground or on the plants and would shelter in drought-induced cracks of the soil when troubled. Specimens collected on August 4th and taken back to the laboratory for further imaging (Figure 1C) were copulating at high frequency.

In the same pools imagos were no longer observed after August 10th until the end of September. Two adults, apparently freshly emerged, were found, however, in trap bags surrounding **Cressa** plants on September 27th. Finally, on November 17th 1999, beating and sweeping the **Cressa** plant emerging from the water produced two more imagos.

Beyond the 1999 campaign, we consistently observed the recurring presence of **Sharpia rubida** attacking **Cressa cretica** populations in years 2000, 2002, 2007, 2008. Occasionnally, searches for adults when the marshes are flooded led us to find **Sharpia** imagos over-wintering in the cracks of barks on the branches of ***Tamarix*** trees growing near the marshes (10 imagos at Baisse des Tirasses in October 2002).

Moreover, in the course of a broader regional survey along the French Mediterranean coast, **Sharpia rubida** was found in several other pools associated with **Cressa**: Hérault, Frontignan (03°45'E 43°26'N) 30 VIII 2000, 1 imago and eggs in flowering buds; Hérault, Vic-la-Gardiole (03°48'E 43°29'N), 30 VIII 2000, destroyed flower buds; Vaucluse, Bonnieux (05° 18'E 43°49'N), 31 VIII 2000, 1 imago; Corsica, Barcaggio (09°22'E 42°41'N), 3 X 2000, 1 imago.

Survey of the flower buds

Out of a total of 7,515 flower buds of **Cressa cretica** examined from the ramets sampled, the rate of destroyed buds observed at the top of the infestation ranged between 69% and 97% (mean: 89.8%, s.d.: 8.2) with no significant difference between years (Table 1). Similarly, the mean rate of aborted buds per site (8.9% +/- 7.8) did not differ significantly between years. In contrasts more buds reached the flower stage in 1999 than in 1998: only 1 site produced flowers

(4% of buds at Baisse des Tirasses), 1 site did not show any flowering buds and in the other sites all buds were destroyed before the flowering stage (Table 1). In 1999, larges differences in flowering rates were observed with 18.1% of buds in Cerisières-South when in the other sites the values ranged between 0 and 2.1% (Table 1). No fruits were observed on the sample of ramets in 1999 while only few occurred at Baisse des Tirasses (0.2%) in 1998.

Each flowering bud hosted a single larva at most, which would eat the entire ovary throughout its development.

Depending on the station, the rate of destroyed buds observed at the top of the infestation ranged between 85% and 96%. This maximum rate was found on July 27th and August 09th 1999. The percentage of larvae actually present at the time of observation, however, only ranged from 5% to 15% of buds (in 1999). In most of the buds assessed as "parasited" the larvae had already left the remaining parasited buds.

"Rain" of larvae

In the traps installed below **Cressa** plants in year 2000 on Baisse des Tirasses, fourth-instar larvae were captured in 224 h distributed between July 21st and August 87th (Fig. 3), with a cumulated calculated density of 654 individuals/m^2 (Fig. 2).

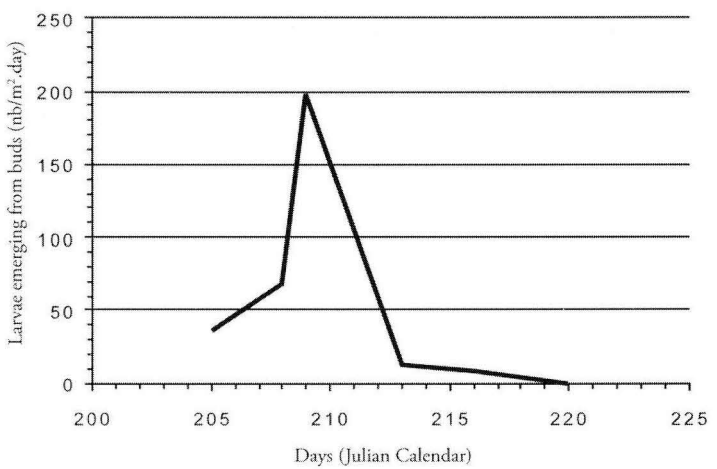

Figure 3. Distribution of larvae dropping from flower buds of **Cressa cretica** per day (caught in traps, see Methods) between July 23rd (Day: 205) and August 8th (Day: 220) 2000 on Baisse des Tirasses.

The time needed in laboratory between the dropping of the larva from the plant to the hatching of the adult ranged from 22 to 41 days (mean: 28.7± 4.3 days).

Presence of the larvae in the soil

The soil-washing carried-out on August 10th 1999 in the station Cerisière-South yielded 9 specimens of fourth-instar larvae (see methods for larval staging). No larva was found in the following soil-washing between August 26th and October 14th, suggesting that **Sharpia rubida** does not overwinter as a buried larva.

On the same site, in the random sediment cores collected over 0.08 m^2, on 8 August 2000, we sampled 3 larvae, 99 nymphs and 3 pharate adults, which corresponds to a total density 1,230 individuals/m^2.

Description of the fourth-instar larval stage

This description follows the nomenclature of ANDERSON (1947) and MAY (1994). Last larval instar specimens of **Sharpia** measure around 2.5 mm (Figure 2A). However, the elasticity of the body makes this measure variable and this value should only be considered a rough estimate.

Head (Figure 2B). Head free. Frontal suture does not reach the mandibles; endocarinal line absent; setae *des3* on epicranium; 5 pairs of frontal setae (*fs*); frontoclypeal and clypeolabral suture both distinct; 3 pairs of clypeal bristles; Antennae exposed, visible in facial view, as long as wide; 2 ocelli present; mandibles: mola undevelopped, incisor with a single tooth; labrum transverse with at least 8 pairs of setae; maxillary palp 2-segmented, palpiger absent; labial palp 1-segmented.

Thorax. Pronotal shield simple; spiracle (Figure 2C) on prothorax; spiracle canals pointing dorsally; legs absent.

Abdomen. 8 abdominal spiracles, located on pleurum; each spiracle with a single canal pointing posteriorly (Figure 2C); 2 dorsal transverse folds per segment; ventro-pleural lobes complete; abdominal segments VIII and IX simple, not sclerotised; abdominal segment X (anus) simple (not sclerotised) and terminal (not subdorsal or ventral).

Discussion

We have described here the main aspects of the biology of **Sharpia rubida**, a poorly known Mediterranean weevil. The sporadic distribution of its host-plant, **Cressa cretica**, confined to an ecologically peculiar habitat explains largely the lack of information on this species. We may expect, however, that the recent spreading of this plant in France results in an expansion of **Sharpia rubida**. Consistent with this, **Sharpia rubida** was recently observed in Corsica, Provence and Languedoc in 5 locations, and indirect evidence of its presence

was found in an additional site. It is likely that the species has been overlooked in several of the visited sites, especially in Corsica where the dates of visit were late.

The clearest evidence of the presence of **Sharpia rubida** lies in the low flowering rate of **Cressa cretica** populations. The destruction rate of the flower buds (before flowering) of **Cressa cretica** by **Sharpia rubida** was found to be very high (around 90%), even if the level of parasitism *sensu stricto* (larvae found in the bud) did not seem to exceed 15%. The remaining destroyed buds could result from adults feeding on them, failed oviposition, or aborted larval development.

The impact of **Sharpia rubida** on the reproduction of **Cressa cretica** is tremendous with only a very limited fraction of the flower buds reaching the flower stage. The rate of destruction measured showed some variance between sites and years that suggests that in some years the flowering rate can be more important. The occasionally observed extensive blooming of flowers of **Cressa cretica** suggests that populations of **Sharpia rubida** experience large fluctuations in size, allowing a successful seed yield in some years. The massive flower bud destruction does not, however, appear to harm the populations of **Cressa cretica.** The recent expansion of the **Cressa cretica** species on new sites on Tour du Valat estate suggests that propagules are readily available and efficiently dispersed. More generally the species is expanding in the French Mediterranean region. **Cressa cretica** is a perennial species that does not need to produce seeds every year to maintain its populations and shows an extensive vegetative growth. Yet the causes of fluctuations of populations of **Sharpia rubida** are not known. Large inter-annual fluctuations in the hydrological regime of the temporary pools could play an important role. The early flooding of the pools could have a detrimental impact on the populations of **Sharpia** if the adults have not yet emerged from the sediment. Furthermore, some populations of **Cressa cretica** do not enter into reproduction (dry year) preventing the reproduction of **Sharpia rubida**. Finally, the phenology of the reproduction of **Cressa cretica** seems variable between years and sites (unpublished material) ranging from mid-July to late August in the Camargue which could affect the reproduction of **Sharpia rubida**. The date of drying up of the pool is probably a key trigger for the phenology of the plant which does not stand surface water.

In summary, the life cycle of **Sharpia rubida** could be outline as follow: The larval development occurs in flower buds, where female **Sharpia** lay a single egg. The **Sharpia** larva consumes the flower bud's ovary entirely. The fourth-instar larva leaves the empty bud and drops to the ground to undergo metamorphosis in the soil near the plant by the end of the summer. About a

month later, shortly before the marshes get flooded, the imago emerges and moves to a nearby shelter such as the bark of a ***Tamarix*** branch to overwinter. Adults will become active again in the following summer, when the **Cressa** plants develop again. Therefore, the complete development from egg to adult is short, in the range of two to three months (from late July to late October).
The absence of buried larvae in the late summer and the fall suggests that the metamorphosis occurs in a matter of weeks after the fourth-instar larva leaves the flower bud. This is consistent with the presence of (presumably freshly emerged) adults clustering under ***Tamarix*** barks in November.

Some aspects of the biology of **Sharpia rubida** await more information, however, including a better quantification of relative amount of parasitism over predation, but also a more complete description of the larval development, and in particular the identification and description of the nymph. Finally, these conclusions rely on a dataset obtained at the far North of the species' geographical area, and a generalisation is premature, in particular in respect to the timeline of its life cycle. Preliminary observations suggest possible variations between sites or years in the life-cycle, or both, early imaginal stages being observed in late August (e.g. one fresh imago found at Bonnieux, near Martigues on 31st August 2000).

We hope to provide enough information here for other naturalists to make new observations elsewhere in order to increase our knowledge of the couple **Cressa cretica** / **Sharpia rubida**. A detailed mapping of both species, along with indications on the reproduction mode of **Cressa cretica** in the presence or absence of its predator will provide ground to measure the selective pressure of **Sharpia rubida** on its host-plant.

Acknowledgements

The authors want to thank Thibaut Bourse for his cheerful help in the field regardless of the mosquitoes and the mud, Guilhan Paradis for a field tour of **Cressa** populations in Corsica. We are also grateful to Christian Cocquempot and Michel Martinez (INRA, Montpellier) for granting us access to the insect collection of the INRA/ENSA-M and to the Conservatoire Botanique National de Porquerolles for providing us with information on populations of **Cressa cretica** in France.

References

ALONSO-ZARAZAGA, M. A., SAN VICENTE, I. U. y COELLO, P., 2009. Nuevos datos sobre la distribución de Sharpia rubida (Rosenhauer, 1856) (Coleoptera,

Curculionidae, Smicronychini). *Boletín de la Sociedad entomológica aragonesa,* **44**: 573-574.

ANDERSON, W. H., 1947. A terminology of the anatomical characters useful in the taxonomy of weevil larvae. *Proceedings of the entomological Society of Washington*, **49**: 123-132.

AUSTIN, D.F., 2000. A revision of *Cressa* L. (Convolvulaceae). *Botanical Joumal of the Linnean Society*, **133**: 27-39.

BEDEL, L., 1888. *Faune des Coléoptères du Bassin de la Seine - **VI***: 385-442 *Rhynchophora*. Annales de la Société Entomologique de France (hors série).

CAILLOL, H., 1954. *Catalogue des coléoptères de Provence (4ème partie). **IV***. Muséum d'Histoire naturelle (Entomologie agricole), Paris (p. 215).

HOFFMANN, A., 1958, *Faune de France. Vol. 62, Coléoptères Curculionidae, troisième partie.* Librairie de la Faculté des Sciences, Paris, 1958 (pp. 1408-1409).

HUSTACHE, A., 1930. Curculionidae Gallo-Rhénans. *Annales de la Société Entomologique de France*, **99**: 198-199.

LAVAGNE, A., 1995. *Cressa cretica* L. in: L. Olivier, et al., Eds., 1995. *Livre rouge de la flore menacée de France. Tome 1 : Espèces prioritaires*. Muséum National d'Histoire Naturelle - Service du Patrimoine Naturel - Conservatoire Botanique National de Porquerolles - Ministère de l'Environnement - Direction de la Nature et des Paysages, Paris, 486 p.

MAY, B.M., *An introduction to the immature stages of Australian Curculionoidea. In: E. C. Zimmerman, (Ed.), Australian Weevils (Coleoptera: Curculionoidea). Volume II. Brentidae, Eurhynchidae, Apionidae and a Chapter on Immature Stages by Brenda May*. CSIRO Publishing Canberra, Australia, 1994 (pp. 365-728).

OSELLA, G. and DI MARCO, C., 1996. Nuovi Curculionoidea della fauna Siciliana (Coleoptera). *Bollettino del Museo Civico di Storia Naturale di Verona,* **20**: 345-370.

PIGNATTI, S., 1982. *Flora d'Italia*. Edagricole, Bologna.

THEROND, J., 1976. *Catalogue des coléoptères de la Camargue et du Gard (2ème partie)*. Société d'étude des sciences naturelles de Nîmes (pp. 174-175).

TOURNIER, H., 1874. Matériaux pour servir à la monographie de la tribu des Erirrhinides, Famille Curculionidae. *Annales de la Société Entomologique de Belgique*, **17**: 63-116.