

Host Specificity of an Italian Population of *Cosmobaris scolopacea* (Coleoptera: Curculionidae), Candidate for the Biological Control of *Salsola tragus* (Chenopodiaceae)

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Summary

Russian thistle, *Salsola tragus* L. (Chenopodiaceae) is a troublesome weed infesting the drier regions of western North America. It is native to Central Asia and infests rangelands and semi-arid pasture lands, croplands, residential, disturbed and industrial areas. *Cosmobaris scolopacea* (Germar) is a weevil distributed in Eurasia and North America, and generally associated with plant species of the family Chenopodiaceae. The larvae feed and pupate within the stems of the host plant, and the adults emerge in the following late spring. From preliminary host range testing carried out at the ENEA-BBCA facilities in Rome, Italy, it appeared that *C. scolopacea* might harbour different host races, one being potentially more specific to the target and only present in Sicily, Italy. To determine species boundaries and reveal population structure at the intraspecific level, a phylogeographic study using the mitochondrial COI gene was conducted on specimens collected in the native range (Italy, Spain, Iran, Bulgaria, Turkey) and the U.S.A. The study confirmed the presence within the species of a highly divergent Sicilian lineage that has only been reared from *Salsola kali* L. The degree of specificity of this particular lineage and hence host race status is being tested through host specificity testing. Preliminary results seem to indicate that this Sicilian lineage can be at least a true *Salsola* host race, opening doors for further testing as a biological control agent for Russian thistle.

Introduction

Salsola tragus L. (*sensu lato*), together with other closely related Russian thistle species, is a troublesome weed in the drier regions of western North America (Young, 1991). It infests range and semi-arid pasture lands as well as cropland, agricultural, residential and industrial areas. As a crop weed it can cause

yield losses of greater than 50% in spring wheat (Young, 1988). It is also a host for several crop pests, and the tumbling plant skeletons can fill irrigation canals and pile against fences (Goeden and Ricker, 1968). A biological control program started during 1970s and there is still a need for effective agents (Goeden and Pemberton, 1995; Smith et al., 2006).

Larvae of *Cosmobaris scolopacea* (Germar,

1819) (Coleoptera: Curculionidae) were recorded and reared from plants of *Salsola kali* L. (Chenopodiaceae) near Catania (Sicily, Southern Italy) by Gaetano Campobasso (pers. comm., 2000). Despite the fact that the stem boring weevil is known as a cosmopolitan pest species of several Chenopodiaceae crops, a screening for the evaluation of the host range of this population was started in the early 2000s by G. Campobasso, performing mainly open field host range observations by dissecting native and crop Chenopodiaceae occurring in sympatric conditions with the target weed.

Starting in 2008, we decided to continue the screening of the weevil, by the combination of three different approaches: morphological taxonomy, genetic analysis and ecological bioassays. The purpose of the present study was to compare for these two last aspects different populations of *C. scolopacea* to reveal the existence of cryptic species and /or host races within the species, and to determine if any are potentially suitable as a biological control agent for the target weed.

Material and methods

Field sampling

Weevil larvae were collected from *Salsola* spp. in Eurasia, i.e. Sicily, Central Italy, Central Spain, Central Turkey, Bulgaria, Iran, and in the US (California) from 2008 to 2010. In most of the sites, larvae were collected also in the stems of other Chenopodiaceae (often *Chenopodium album* L. and rarely on *Halimione* spp.). Stem dissection was conducted *in situ*, with some of the larvae preserved in absolute ethanol for genetic studies and others transferred to artificial diet (Tomic-Carruthers, 2009) for adult emergence to use for morphological study. Adults have been collected in two locations in Sicily (Simeto and Eraclea Minoa, respectively) to carry out host range tests in laboratory conditions at the BBKA facilities.

Molecular and phylogenetic analysis

Weevils were collected as adults and larvae from a total of i) 29 populations throughout the Eurasian native distribution range from Italy to Northern

Iran and ii) 2 populations in North America, and iii) across three major host plants in the Chenopodiaceae family, i.e. the Russian thistle (*Salsola* spp.), *C. album* and *Halimione* spp. Also included in this study as an outgroup was a dried specimen of *Cosmobaris discolor* (Boheman, 1836) collected by E. Colonnelli in South Africa in 2007 on *Chenopodium* sp. Weevils were preserved in absolute ethanol and stored at -20°C before DNA extraction. Genomic DNA was extracted from single specimens using either the CTAB protocol (Doyle and Doyle, 1987) or the DNeasy Blood and Tissue DNA extraction kit (Qiagen S.A, Courtaboeuf, France) following the manufacturer's protocol. A ~830 bp section of the mitochondrial cytochrome oxidase c subunit I (COI) gene was amplified through Polymerase Chain Reaction (PCR) in a 9700 Perkin Elmer thermal cycler (Applied Biosystems) using primers C1-J-2183 and TL2-N-3014 (Simon et al., 1994) and PCR profile: 5 min at 94°C, 5 cycles of 30s at 92°C, 30s at 48°C, 1 min at 72°C, followed by 25 cycles of 30s at 92°C, 30s at 52°C, 1 min at 72°C, and 7 min at 72°C. PCR products were sequenced on both strands at Genoscreen (Lille, France) on ABI 3130XL automatic sequencers (Applied Biosystems, Foster City, CA, USA). Alignments of consensus sequences were manually edited with Bioedit 7.09 (Hall, 1999). A dataset of 79 sequences of 638bp of length was obtained for *C. scolopacea* sensu lato. To determine species boundaries and reveal discontinuities among lineages at the intraspecies level, an analysis method based on haplotype relationships (i.e. statistical parsimony) was chosen (TCS; Clement et al., 2000). Under the 95% parsimony criterion haplotype network resulted in three unconnected networks (data not shown). To provide a framework for understanding the evolutionary relationships between all populations and between these haplotype networks, a phylogenetic analysis was performed on the same dataset. Modeltest version 3.7 (Posada and Crandall, 1998) was used to determine the model of nucleotide substitution that fitted the data best. The hierarchical likelihood ratio (hLRT) test as implemented in Modeltest selected the HKY+G model as the best fit for our dataset. The Maximum Likelihood analysis was conducted under PhyML 3.0 (Guindon et al., 2010), and bootstrapping was calculated from 100 replicates. Genetic divergence levels within and between networks and species were

determined by calculating un-corrected p distances in PAUP*4.0 (Swofford, 2002).

Host range experiments

Laboratory host range choice-tests were carried out during 2010, testing one population from *Eraclea Minoa*, Western Sicily. Bioassays were carried out in Petri dishes in a climatic cabinet at 21-26°C and with a 14:10 h L/D cycle, confining one female with one stem of *S. kali* (SAKA) plus one stem of one of the following plant species: *Kochia scoparia* (L.) Schrader (KOSC), *Chenopodium album* (CHAL), *Suaeda taxifolia* (P. C. Standley) P. A. Munz (SUTA) and *Bassia hyssopifolia* (Pallas) Volk (BAHY).

Results and Discussion

The ML phylogenetic tree obtained on the basis on the 79 COI sequences of *C. scolopacea* sensu lato that was rooted with *C. discolor* as an outgroup is presented in Figure 1. Three major highly diverged mitochondrial clades supported with high bootstrap values above 93% can be observed, and are equivalent to the three unconnected haplotype networks obtained under TCS. One clade (A) contained the nine North American specimens from one site in California and one site in Prince George's County, Beltsville, Maryland, corresponding to two haplotypes. Specimens from *S. tragus* and *C. album* at the Brentwood, CA site shared the same haplotype. Genetic divergence within this clade was very low (0.06%). A second clade (B), also named the Sicilian clade, contained the 18 samples collected in Sicily and that were associated with *S. kali* and *Halimione* sp. hosts. It contained three haplotypes, and its

intraclade genetic divergence averaged 0.25%. The third clade (C) contained the remaining 52 samples distributed across Eurasia and that were associated with *Salsola* and *Chenopodium* host plants. A total of 24 haplotypes belong to this clade which has the highest intraclade genetic divergence among the three, averaging 0.56%. The genetic divergence between the three clades ranged from 8.5% between the "American" clade (A) and the "C" clade to 9.2% between the Sicilian clade (B) and the "C" one. These values are nearly of the same order as those observed between the outgroup species *C. discolor* and any of the three clades. First described by Casey in 1920, the American lineage has been assumed to be *C. americana* Casey (Casey, 1920; O'Brien and Wibmer, 1982). In the present day, from gathered morphological data, it is admitted that *C. americana* Casey is a synonym of *C. scolopacea* and should not retain its separate name (Colonelli, pers com.). However, to determine whether the extent of the divergence is sufficient for the three clades to be considered cryptic species, sub-species, host races or biotypes, further research is likely required in the future.

The presence of a highly divergent lineage of *C. scolopacea* in Sicily that was collected mainly on *S. kali* supported Campobasso's hypothesis that Sicilian populations may be more host specific than other populations of *C. scolopacea* in Eurasia. We therefore conducted host range testing of specimens from the Sicilian clade, collecting individuals as adults on *S. kali* (the Sicilian subclade highlighted in grey in the ML tree). Two-way choice oviposition experiments carried out during 2010 in Petri dishes showed a clear preference of the weevil for *S. kali*, with occasional oviposition on *B. hyssopifolia*, *C. album* and *S. taxifolia* (Table 1).

Table 1. Oviposition preference by *Cosmobaris scolopacea* in two-choice tests.

| Plant spp. ¹ A+B | No. of reps | eggs on A (mean) | Std. Error | eggs on B (mean) | Std. Error | Wilcoxon Signed Ranks Test (Z) | Asymp. Sig. (2 tailed) |
|--------------------------------|----------------|---------------------|------------|---------------------|------------|--------------------------------------|---------------------------|
| SAKA+BAHY | 7 | 2.00 | 0.76 | 0.57 | 0.43 | -1.279 | 0.201 |
| SAKA+KOSC | 6 | 3.00 | 0.82 | 0.00 | 0.00 | -2.226 | 0.026 |
| SAKA+CHAL | 6 | 2.33 | 0.61 | 0.17 | 0.17 | -2.214 | 0.027 |
| SAKA+SUTA | 9 | 1.78 | 0.43 | 0.11 | 0.11 | -2.354 | 0.019 |

¹BAHY = *Bassia hyssopifolia*, CHAL = *Chenopodium album*, KOSC = *Kochia scoparia*, SAKA = *Salsola kali*, SUTA = *Suaeda taxifolia*.

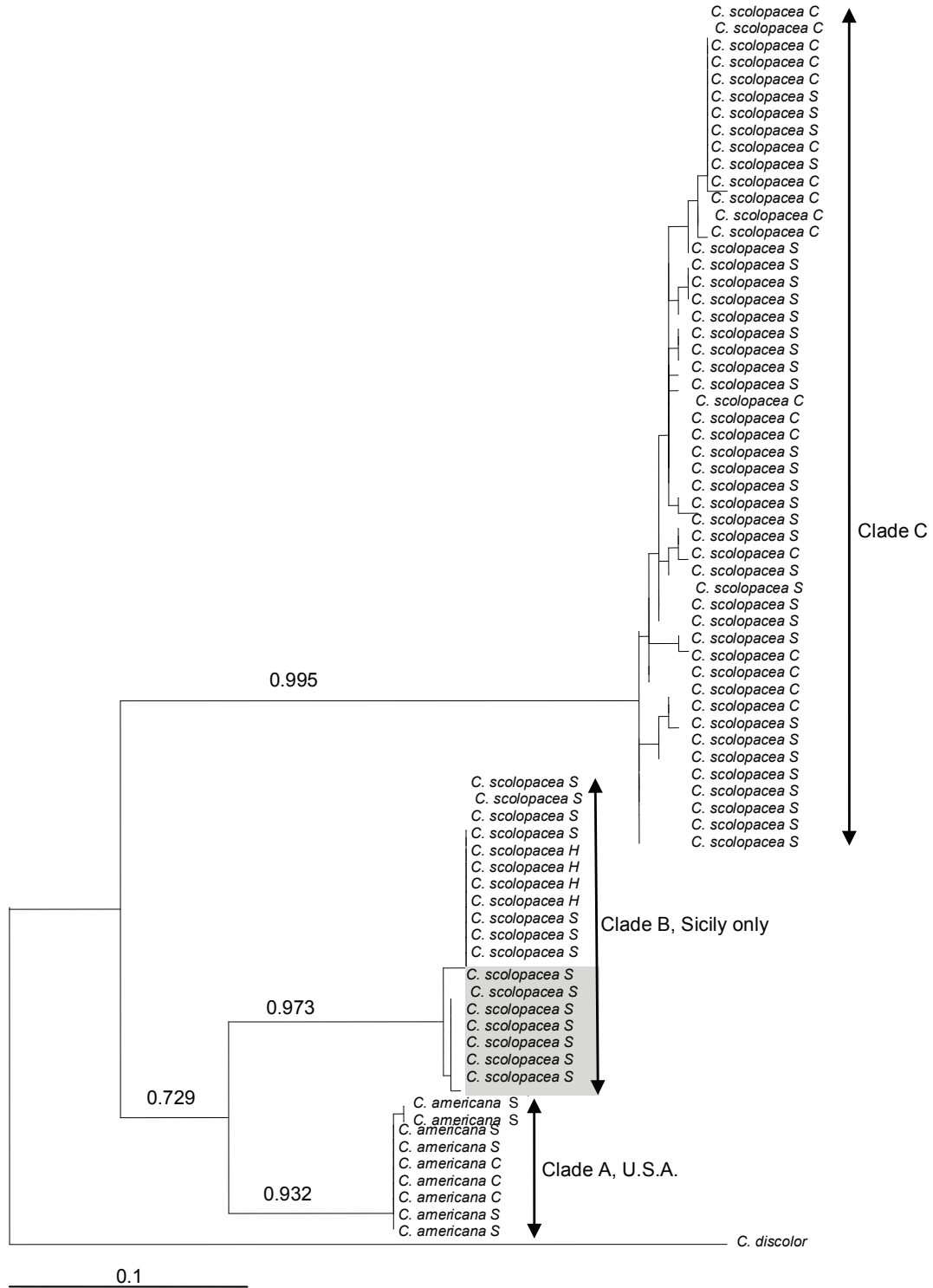


Figure 1. Phylogenetic tree inferred by the maximum likelihood method based on mitochondrial COI sequences of various populations of *Cosmobaris* spp. Bootstrap scores (100 replicates) are indicated along the branches. The letters following the sample names refer to the host plants (S: *Salsola* spp.; H: *Halimione* spp.; C: *Chenopodium album*). The weevil populations used for the host specificity testing were from the “Sicilian” sub-lineage (gray-shaded block). The scale bar below the ML tree indicates the number of substitutions per site.

As recently and extensively reviewed by Gaskin et al. (2011), for weed biological control practitioners, there is a reasonable consensus that specialized lineages, including morphocryptic ones, whatever the stage of speciation they represent, are now considered one of the best routes for efficacy as regards their host specificity and perhaps safety. Hence, depending upon future results, the *Cosmobaris* weevil could be yet another example of phytophagous weevils that have several host races or cryptic species, some being strictly specific to a targeted weed, and hence opening the door for potential use in biological control (Fumanal et al., 2004; Antonini et al., 2008; Gaskin et al., 2011).

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