

# Insights into the subgenera *Chrysolina* s. str. and *Rhyssoloma* Woll. of *Chrysolina* Mots. (Coleoptera: Chrysomelidae: Chrysomelinae)

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**Abstract** - The taxonomic position of *Chrysolina costalis* and *C. wollastoni* in the subgenera *Chrysolina* s. str. or *Rhyssoloma* is ambiguous. Recently, they have moved from the former to the latter subgenus, by taking four morphological characters. However, *C. (Rhyssoloma) fragariae*, the type species of this subgenus, is clearly distinguished with regard to the two previous species, from other morphological features, also the male karyotype of  $2n=24(Xy_p)$  instead of  $2n=23(X0)$  chromosomes, and mainly in the DNA molecular phylogenetic trees where *C. costalis* and *C. wollastoni* appear in the same highly supported clade with *C. bankii*, another species of the *Chrysolina* s. str. subgenus. Therefore, the subgenus *Rhyssoloma* should be maintained as monotypic with *C. fragariae* as its unique species like it was before.

**Key words:** Chrysomelidae, *Chrysolina* s.str., *Rhyssoloma*, subgenera, species.

**Riassunto** - Approfondimenti sui sottogeneri *Chrysolina* s. str. e *Rhyssoloma* Woll. di *Chrysolina* Mots. (Coleoptera: Chrysomelidae, Chrysomelinae).

La posizione tassonomica di *Chrysolina costalis* e *C. wollastoni* nei sottogeneri *Chrysolina* s. str. o *Rhyssoloma* presenta ambiguità. Recentemente i due taxa sono stati trasferiti dal sottogenere nominale a *Rhyssoloma* sulla valutazione di quattro caratteri morfologici. Tuttavia, *C. (Rhyssoloma) fragariae*, specie tipo del sottogenere, si distingue chiaramente dalle due specie citate, oltre per caratteri morfologici, per avere il cariotipo del maschio di  $2n=24(Xy_p)$  anziché di  $2n=23(X0)$  cromosomi e, principalmente, per l'albero filogenetico DNA molecolare in cui *C. costalis* e *C. wollastoni* compaiono nello stesso clade fortemente supportato, insieme a *C. bankii*, specie già inclusa nel sottogenere *Chrysolina* s. str. Conseguentemente il sottogenere *Rhyssoloma* deve essere mantenuto come monotipico per la sola *C. fragariae*, così come considerato finora.

**Parole chiave:** Chrysomelidae, *Chrysolina* s.str., *Rhyssoloma*, sottogeneri, specie.

## INTRODUCTION

Bienkowski (2019) published the most recent and highly valuable taxonomic review on the genus *Chrysolina*

na Motschulsky 1860 where 430 species of all described 70 subgenera are dealt with, plus 59 species of unnamed groups, among these subgenera, *Chrysolina* s. str. is composed of four species and *Rhyssoloma* of three other ones. This treatment is in conflict with previous views (Bourdonné & Doguet, 1991; Bienkowski, 2001; Kippenberg, 2010), because two species of the former subgenus, *C. costalis* (Olivier 1807) and *C. wollastoni* (Bechyné 1957) have been moved to *Rhyssoloma* (Bienkowski 2019). Thus, this latter subgenus would include *C. costalis*, *C. wollastoni* and *C. fragariae* (Wollaston 1854), contrary to the ancient *Rhyssoloma* as a monotypic subgenus, with a unique species, *C. fragariae*. We shall analyze these two taxonomic alternatives in order to see which of them is the most likely.

## RESULTS

According to Bienkowski (2019, p. 757), the subgenus *Rhyssoloma* is defined by four morphological characters: 1) prothoracic hypomeron without distinct impression, wrinkles and lateral border, 2) elytral punctures arranged in 11 regular paired rows, 3) claw tarsomere without distinct denticles but with its apical margin projected, and 4) pygidium with a broad longitudinal impression in basal 1/2 - 2/3 only. Nevertheless, at least in *C. wollastoni*, the elytral punctures are quite irregular, very weak, and in the two other species only those punctures of elytral disc are regular but not the others.

Conversely, taking into account morphological characters too, 1) *C. fragariae* shows an outstanding relief in lateral sides of each elytron, which are also impressed and irregularly wrinkled, contrary to those without them shown in *C. costalis* and *C. wollastoni*, 2) also *C. fragariae* shows the third maxillary palpomere not broader than the second in contrast with the former species, 3) it has not a setiferous pore in pronotum which is present in the two other species, and 4) its aedeagus lacks the flagellum contrary to the other species. Furthermore, although the three species are Atlantic Macaronesian endemics, *C. fragariae* lives in Madeira island solely, whereas *C. costalis* and *C. wollastoni* are only living in Canary Islands.

The genetic data are strikingly in agreement with these morphological differences. The male karyotype of *C.*

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*fragariae* has  $2n=24$  chromosomes and a meioformula of  $11+Xy_p$ , which means that both male sex-chromosomes, a large or medium-size X and a tiny y-chromosome are associated at first meiotic metaphase in a “parachute-like  $Xy_p$ ” configuration (Petitpierre, 1988), while *C. costalis* (sub *C. obsoleta* Woll.) and *C. wollastoni* (sub *C. rutilans*) have a male karyotype of  $2n=23$  chromosomes and a meioformula of  $11+X0$ , due to the absence of y-chromosome (Petitpierre, 1975, 1981, 1983). In addition, the three cytogenetically studied of the four species belonging to the ancient subgenus *Chrysolina* s. str., *C. bankii* (Fabricius 1775), *C. rufa* (Duftschmid 1825) and *C. staphylaea* (Linnaeus 1758), share the same male karyotype and meioformula,  $2n=23$  chromosomes and  $11+X0$  (Petitpierre, 1975, 1981, 1983; Barabás & Bezo, 1979). This male karyotype of  $2n=23(X0)$ , has only been found in another subgenus and species among the total of 72 chromosomally sampled taxa of *Chrysolina* (Petitpierre, 2011), that of *C. (Lithopteroides) exanthe-matica* (Wiedemann 1821), though this subgenus and its unique species is very different from all the other known subgenera of *Chrysolina* (Bienkowski 2019).

Even with a much more genetic accuracy than karyotypes, the molecular phylogenies based on the mitochondrial 16S rDNA (*rrnL*) sequences and mitochondrial cytochrome oxidase subunit 1 (*coxI*) DNA ones (Garin *et al.*, 1999), joins *C. bankii* and *C. costalis* (sub *C. obsoleta*) in a well-supported clade. Likewise, Gómez-Zurita *et al.* (1999) proved the close phylogenetic interrelationships of *C. bankii*, *C. costalis* (sub *C. obsoleta*) and *C. wollastoni* (sub *C. rutilans*), by analyzing a 510 bp fragment of 16S rDNA (*rrnL*) sequences. Finally, in the last phylogenetic study using these two mitochondrial gene fragments and a third of a nuclear histone 3 gene (*H3*), both the Maximum Likelihood and the Bayesian phylogenetic trees confirmed and substantiated again the above mentioned results (Jurado-Rivera & Petitpierre, 2015).

## DISCUSSION AND CONCLUSIONS

The four anteriorly described morphologic characteristics of *C. fragariae*, mainly its outstanding relief in each elytron, and the  $2n=24$  chromosomes and  $11+Xy_p$  male meioformula, clearly separates the subgenus *Rhyssoloma* from the subgenus *Chrysolina* s. str. whose species lack these elytral reliefs, share  $2n=23$  male chromosomes and  $11+X0$  male meioformula. In addition, the diverse molecular phylogenetic trees obtained from the sequences of DNA fragments, either mitochondrial, nuclear, and both combined gene sequences have strikingly demonstrated that the three analyzed species of *Chrysolina* s.str. subgenus, *C. bankii*, *C. costalis* and *C. wollastoni* belong to a highly supported clade, although *C. (Rhyssoloma) fragariae* has not been phylogenetically analyzed yet. With the present state of knowledge, we have no reason to move *C. costalis* and *C. wollastoni* from the *Chrysolina* s. str. to the *Rhyssoloma* subgenus as held before by all authors (Bourdonné & Doguet, 1991; Bienkowski, 2001; Kippenberg, 2010), prior to the change of opinion proposed by Bienkowski (2019).

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