

1 Widespread presence of *Wolbachia* in an Alpine population of the viviparous leaf
2 beetle *Oreina cacaliae* (Coleoptera: Chrysomelidae)

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12 **Key words:** insect symbiosis, 16S rRNA, microbiota, reproductive strategies.

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14 **Abstract**

15 *Oreina cacaliae* (Coleoptera: Chrysomelidae) is a rare example of viviparous insect,
16 able to feed on toxic plants and sequester toxic compounds. Here we present the
17 results of a study on the microbiota associated with *O. cacaliae*, based on 16S rRNA
18 bacterial gene sequencing. *Wolbachia* resulted as the dominant bacterium, both in
19 males (100%) and in females (91.9%). Based on multilocus sequence typing, the
20 detected *Wolbachia* was described as a new sequence type (*Wolbachia*
21 *Ocac_A_wVdO*). Phylogenetic analyses assigned *Wolbachia Ocac_A_wVdO* to
22 supergroup-A. In situ hybridization and electron microscopy confirmed the presence
23 of *Wolbachia* within *O. cacaliae* oocytes, indicating its transovarial transmission.
24 PCR specific for *Wolbachia* was performed on representatives of six species of
25 *Oreina*; the presence/absence of *Wolbachia* was then mapped on a cladogram
26 representing the phylogeny of the insect host. Finally, since viviparous species of
27 *Oreina* were either infected or non-infected by *Wolbachia*, we cannot derive any
28 conclusion about the possibility that this symbiont played some role in the evolution
29 of viviparity.