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Keywords:	<i>Cacyreus marshalli</i> , native natural enemies, <i>Trichogramma brassicae</i> , <i>Exorista larvarum</i> , <i>Brachymeria tibialis</i> , biological control

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SHORT COMMUNICATION (no. Words 2230 except Title, Abstract, Tables and captions)

**Acceptance and suitability of *Cacyreus marshalli* (Lepidoptera: Lycaenidae) as host
for three indigenous parasitoids**

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21 **Abstract:** Laboratory tests were conducted in Italy to evaluate the acceptance and suitability of
22 the alien butterfly *Cacyreus marshalli* Butler as host for three indigenous parasitoids,
23 *Trichogramma brassicae* (Bezdenko) *Exorista larvarum* (L.) and *Brachymeria tibialis* (Nees).
24 Only *E. larvarum* and *B. tibialis* showed potential to adapt to *C. marshalli*. Their
25 contribution to biological control appeared to be especially related to host mortality due
26 to incomplete parasitoid development.

27

28 **KeyWords:** *Cacyreus marshalli*, native natural enemies, *Trichogramma brassicae*,
29 *Exorista larvarum*, *Brachymeria tibialis*, biological control

30

31 The Geranium Bronze *Cacyreus marshalli* Butler, native of South Africa, has established in
32 Italy and other European countries (Suffert 2012). In South Africa *Apanteles* sp., other unidentified
33 braconids and small tachinids were reported as larval parasitoids of *C. marshalli* (Clark and
34 Dickson, 1971). In Europe, two native parasitoids have been recorded as antagonists of this exotic
35 pest of cultivated geraniums, namely the hymenopteran *Trichogramma evanescens* Westwood in
36 Spain (Sarto i Monteys and Gabarra 1998) and the tachinid *Aplomya confinis* (Fallen) in Italy
37 (Vicidomini and Dindo 2006).

38 With the aim of enhancing knowledge on the associations between *C. marshalli* and
39 indigenous antagonists, laboratory tests were conducted to evaluate the acceptance and suitability of
40 this alien species as host for three polyphagous parasitoids of Lepidoptera, all widespread in Italy
41 and Europe (www.faunaeur.org accessed January 9 2013): the hymenopterous egg parasitoid
42 *Trichogramma brassicae* (Bezdenko), the tachinid larval parasitoid *Exorista larvarum* (L.) and the
43 hymenopterous pupal parasitoid *Brachymeria tibialis* (Walker).

44 Colonies of *C. marshalli*, maintained as described by Quacchia, Ferracini, Bonelli, Balletto,
45 and Alma (2008), and *Ephestia kuehniella* Zeller, used as control, were supplied by DISAFA.
46 Batches of parasitized *E. kuehniella* eggs were supplied by Koppert Biological Systems (The
47 Netherlands).

48 Two experiments were carried out to test the acceptance and suitability of *C. marshalli* by *T.*
49 *brassicae*. *Cacyreus marshalli* eggs were obtained in the first experiment by releasing twenty
50 newly-emerged adults in a cage containing two *P. zonale* potted plants. After 24 hrs portions of
51 leaves with eggs were cut off and exposed to twenty less than 24hrs-old mated and unfed *T.*
52 *brassicae* adults with no previous contact with a host egg . After 48 hrs the leaf portions were
53 individually isolated. Ten replicates (= 10 tubes with 5 eggs each for a total of 50 eggs) were carried
54 out. In the second experiment, three healthy *P. zonale* potted plants were individually exposed to
55 50, 100, and 200 *C. marshalli* (males and females) for 48 hrs. They were singularly isolated and the
56 number of eggs laid by the butterfly was recorded. *Trichogramma brassicae* adults were released in

57 each of the three cages (100, 1,000 and 50,000, respectively). As control, ten replicates of five 72 h-
58 old *E. kuehniella* eggs, distributed on a sticky strip, were performed. Each of these strips was
59 exposed to twenty *T. brassicae* as described above. The eggs were checked daily and the parasitism
60 rate and percentage of emergence for each treatment were estimated.

61 *Exorista larvarum* and *B. tibialis* were maintained on the factitious lepidopterous host
62 *Galleria mellonella* (L.) as described by Dindo, Farneti and Baronio (2001) and Dindo, Marchetti
63 and Baronio(2007). Females had already oviposited on/in *G. mellonella* larvae/pupae before the
64 test. The acceptance and suitability of *C. marshalli* and *G. mellonella* (maintained as control) by
65 the two parasitoids were tested under no-choice conditions.

66 For *E. larvarum*, 60 last instar larvae of each lepidopterous species (Mellini, Gardenghi and
67 Coulibaly 1994), were individually placed in a plexiglass cage containing about 25 parasitoid adult
68 females and 25 adult males, which had emerged 5-6 days before (Dindo et al. 2007). *Cacyreus*
69 *marshalli* and *G. mellonella* larvae were, respectively, 0.93 ± 0.06 cm and 2.4 ± 0.03 cm long
70 (mean \pm SE) when they were tested. The larvae were removed from the cage after 2 hrs and were
71 individually transferred into Petri dishes with food until death, parasitoid puparium formation or
72 host emergence. Each host larva was considered as a replicate. The larvae were deemed to have
73 been “accepted” when at least one *E. larvarum* egg was found on their body. The results were
74 evaluated in terms of the following traits: number and percentage of accepted larvae, eggs/accepted
75 larva, number and percentage of suitable larvae (i.e. accepted larvae from which puparia formed),
76 number and percentage (based on puparia) of emerged flies, number and percentage of dead larvae
77 over accepted larvae, weights of the newly-formed puparia, development times from egg to
78 puparium and from puparium to adult emergence.

79 For *B. tibialis*, *C. marshalli* or *G. mellonella* 2-day old pupae were individually exposed to
80 about 10 parasitoid females and 10 males of mixed ages. The host pupae were removed from the
81 cage as soon as a female pierced their body with the ovipositor and were considered as non-
82 accepted if no ovipositor insertion was detected within 2 hrs. Upon removal from the cage, all

83 pupae were individually kept in plastic Petri dishes until death, parasitoid or host emergence.
84 Twenty-five pupae of each species were tested, each being considered as a replicate. Mean (\pm SE)
85 pupal length was 0.8 ± 0.02 cm for *C. marshalli* and 1.5 ± 0.2 cm for *G. mellonella*. The number and
86 percentage of accepted and suitable pupae (=accepted pupae leading to the emergence of a
87 parasitoid adult) were calculated. The newly-emerged adults were sexed and their weights (in mg)
88 and development times from egg to adult (in days) were separately recorded for males and females.
89 The experiments with the three parasitoids were carried out at $26\pm 1^\circ\text{C}$, $65\pm 5\%$ RH, L16:D8
90 photoperiod.

91 *Trichogramma brassicae* did not parasitize the eggs of the lycaenid, invariably failing to
92 exhibit interest in the hosts. For this reason, no statistical analysis was performed. In the second
93 experiment oviposition by *C. marshalli* occurred on all the tested plants. In particular 219, 421, and
94 630 eggs were recorded on the *P. zonale* plants exposed to 50, 100, and 200 *C. marshalli* adults,
95 respectively. In both experiments no parasitism of *C. marshalli* eggs ever occurred, while in the
96 control the parasitism rate by *T. brassicae* recorded on the factitious host *E. kuehniella* eggs reached
97 a mean value of 92%, with a mean percentage of emergence of 89%. Thus, the commercially
98 produced *T. brassicae* strain evaluated in this study did not prove a good candidate for use against
99 *C. marshalli*. On the contrary, Groussier, Tabone, Coste, and Rizzo (2006) reported good parasitism
100 of *C. marshalli* by *Trichogramma* spp., especially *T. chilonis* Ishii. In view of such positive results
101 and given that many *Trichogramma* species are commonly used as biocontrol agents of various
102 lepidopteran pests (Babendreier, Kuske, and Bigler 2003), the potential of other species different
103 from *T. brassicae* and their role as to host acceptance and suitability deserve further investigation.

104 Conversely, although the mature larvae of *C. marshalli* were considerably undersized
105 compared to the recorded host species of *E. larvarum*, including *G. mellonella* (Cerretti and
106 Tschorsnig 2010), successful parasitism of the lycaenid occurred in the laboratory, but at very low
107 rates (Table 1). *Cacyreus marshalli* larvae were poorly accepted by female flies, possibly due to
108 different factors including their low mobility, an important cue for host acceptance by *E. larvarum*

109 and other *Exorista* species (Stireman 2002; Depalo, Dindo, and Eizaguirre 2012). Suitability to *E.*
110 *larvarum* was also lower for *C. marshalli* compared to *G. mellonella* (Table 1) and, similarly to
111 parasitoid size and development times (reported in Table 2), it was probably affected by host size
112 In this regard, Baronio, Dindo, Campadelli, and Sighinolfi (2002) showed that the development and
113 size of *E. larvarum* were affected both by the amount of food and the vital space available to
114 larvae. Independently of puparium formation, most of the accepted *C. marshalli* larvae died, at a not
115 significantly different rate compared to *G. mellonella*, while the non-accepted ones pupated and
116 emerged as adults (Table 1). As the number of flies obtained from the Geranium Bronze was very
117 low, the puparium-to-adult development times were not subjected to statistical analysis. These
118 times (means \pm SE) were 8.9 \pm 0.2 and 9.5 \pm 0.5 days for the flies respectively obtained from *G.*
119 *mellonella* (n= 36) and *C. marshalli* (n=2), that is slightly longer in the latter host species.

120 All *G. mellonella* and 52% *C. marshalli* pupae were accepted by *B. tibialis* females.
121 Separate 2x2 contingency tables were used to test the independence of host species and number of
122 accepted and suitable pupae. The difference in host acceptance was significant (Yates corrected χ^2
123 =13.27; df=1; P= 0.0003), but the effect of pupal size on this parameter is doubtful since the
124 recorded hosts of *B. tibialis* also include species of similar sizes as *C. marshalli* (Noyes 2012). The
125 percentage of suitable pupae found for *C. marshalli* (=53.8) was lower compared to that recorded
126 for *G. mellonella* (= 84), but the difference was not significant (Yates corrected χ^2 =2.61; df=1; P=
127 0.11). All the accepted pupae of both host species died, whether successfully parasitized or not,
128 while the non-accepted ones (only *C. marshalli*) emerged as adults. All *B. tibialis* which emerged
129 from *C. marshalli* (=7) were males and, as expected, they were significantly undersized
130 (weight=3.8 \pm 0.3 mg) compared to those (=15) obtained from *G. mellonella* (weight=8.4 \pm 0.1 mg)
131 (one-way ANOVA, F= 340.96, df =1, 20; P= 0.0000001). Male development times in *C. marshalli*
132 (=14.9 \pm 0.4 days) and *G. mellonella* (=15.7 \pm 0.3 days) were not significantly different (Kruskall-
133 Wallis test, H= 1.46; N= 22; P= 0.23). The mean weights and development times of the six *B.*

134 *tibialis* females that emerged from *G. mellonella* pupae were 13.8 ± 0.1 mg and 15.7 ± 0.2 days,
135 respectively.

136 The results obtained in the present study suggest that both *E. larvarum* and *B. tibialis* have
137 potential to adapt to *C. marshalli* in nature. Their contribution to biological control appeared,
138 however, to be especially related to host mortality due to incomplete parasitoid development and
139 did not seem to be sufficient to decrease the populations of the target insect pest. A more effective
140 strategy could be represented by classical biological control, with detection and importation of
141 parasitoids of the Geranium Bronze from South Africa to the countries of introduction. A rather
142 recent example of promising classical biological control in Italy is represented by the importation of
143 *Torymus sinensis* Kamijo against *Dryocosmus kuriphilus* Yasumatsu (Quacchia, Moriya, Bosio,
144 Scapin, and Alma 2008). This strategy is not however to be considered as alternative, but rather
145 complementary to the exploitation of indigenous natural enemies. The parasitism of invading novel
146 hosts by native natural enemies has already been reported for a number of various alien insect pests,
147 including *D. kuriphilus* itself (Quacchia et al., 2012), and *Tuta absoluta* (Meyrick) (Ferracini et al.
148 2012). In this context, indigenous natural enemies, including *E. larvarum* and *B. tibialis*, may also
149 play a role in the control of the Geranium Bronze in the countries of introduction.

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152 “New associations between native parasitoids and exotic insects recently introduced in Italy”).

153

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228 **Figure captions**

229

230 Table 1. Acceptance and suitability of *Cacyreus marshalli* and *Galleria mellonella* by *Exorista*
231 *larvarum*: the 2x2 contingency tables for testing the independence of host species and number of
232 A) accepted larvae, B) suitable larvae (= accepted larvae from which puparia formed), C) dead
233 larvae (on accepted larvae); D) puparia which let a fly adult emerge. Yates corrected χ^2 values are
234 given (sample size < 100). Original number of larvae = 60 per species.

235

236 Table 2. Acceptance and suitability of *Cacyreus marshalli* vs. *Galleria mellonella* by *Exorista*
237 *larvarum*: parasitoid eggs per accepted larva, puparial weights and development times from egg to
238 puparium. Means \pm SE. Number of replicates (n) is given in parenthesis above the means. Means in a
239 column followed by the same letter are not significantly different, $P > 0.05$; Kruskal-Wallis test.

240

TABLE 1

Parameter		Host species		χ^2 (df=1)	P
		<i>Galleria mellonella</i>	<i>Cacyreus marshalli</i>		
A)	Accepted larvae (%)	60 (100)	36 (60)	27.55	0.00001*
	Non-accepted larvae (%)	0 (0)	24 (40)		
B)	Suitable larvae (%)	46 (76.7)	13 (36.1)	13.96	0.0002*
	Unsuitable larvae (%)	14 (23.3)	23 (63.9)		
C)	Dead larvae (%)	58 (96.7)	30 (83.3)	3.64	0.06
	Live larvae (%)	2 (3.3)	6 (16.7)		

TABLE 2

Host species	Eggs/accepted larva (no.)	Puparial weight (mg)	Time from egg to puparium (days)
<i>Galleria mellonella</i>	(60) 38.6±3.6a	(72) 30.2±1.6a	(72) 8.1±0.1a
<i>Cacyreus marshalli</i>	(36) 2.4±0.3b	(13) 9.1±0.9b	(13) 8.2±0.6a
H	64.9	28.4	0.15
N	96	85	85
P	0.00001	0.0001	0.69