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# UNIVERSITÀ DEGLI STUDI DI TORINO

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13 **The acoustic repertoire of lycaenid butterfly larvae**

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### 33 **The acoustic repertoire of lycaenid butterfly larvae**

34 Sounds and vibrations play vital roles in intra- and inter-specific communication of many insect  
35 taxa, for sexual display, defence and social recruitment. In Lepidoptera, sound production occurs  
36 in larvae, pupae and adults and has evolved in response to selection of sexual or defensive traits.  
37 About 75% of the 6000 estimated lycaenid butterflies are associated with ants (termed  
38 “myrmecophilous species”) and many species produce acoustic emissions during pre-imaginal  
39 development. It was initially believed that these acoustic emissions were only produced by  
40 myrmecophilous species, but later studies showed that the ability to produce sounds may be  
41 universal among this butterfly family. The acoustic repertoire of the late-instar larvae of 12  
42 lycaenid species (Polyommatainae and Lycaeninae), showing different degrees of interaction with  
43 ants, was analysed by investigating 12 acoustic parameters measured on the call fundamental unit  
44 (pulse). All samples produced species-specific calls whose spectra were characterized by  
45 harmonic frequency components. The inter-specific call diversity better reflects the level of  
46 association with ants than the phylogenetic relationships between species. Our results support the  
47 hypothesis that the ability to emit acoustic signals is widespread in lycaenids, and that these  
48 emissions play a role in myrmecophilous interactions.

49 Keywords: acoustic signals, vibrations, sounds, butterfly, myrmecophily

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51

## 52 **Introduction**

53 Acoustic signals are an extraordinary example of diversity in the animal kingdom. In insects,  
54 vibrational communication conveys both intra- and inter-specific information (Kowalski et al.  
55 2014; Schmidt and Balakrishnan 2015), covering a wide spectrum of purposes (Huber and  
56 Thorson 1985; Hughes et al. 2009; Polidori et al. 2013; Cocroft et al. 2014) and varying in the  
57 ways in which the sound emissions are produced (Drosopoulos and Claridge 2005).

58 Regarding insect sounds, those produced by Lepidoptera were among the first to be studied.  
59 Lepidoptera are known to emit sounds at least since Darwin (1871) described a series of  
60 “clicks” emanated by the nymphalid *Hamadryas feronia*. Since then, acoustic communication  
61 in Lepidoptera has been extensively investigated, with over 200 studies published in recent  
62 decades (Minet and Surlykke 2003), which have primarily focused on hearing and sound  
63 production in adults.

64 Lepidoptera are able to produce substrate-borne vibrations (Yack et al. 2001; Scott et al.  
65 2010) and air-borne signals (Bura et al. 2011) by means of a stridulatory apparatus, tymbal  
66 organs, percussion behaviours or expulsion of tracheal air, from audible frequencies to  
67 ultrasounds (Capinera 2008). Organs that perceive acoustic stimuli have evolved  
68 independently several times within Lepidoptera: in butterflies, tympanal organs can be present  
69 at the base of the forewings, while in moths these sensory organs have been observed in  
70 mouthparts, thorax or anterior abdominal segments (Capinera 2008).

71 Sounds are produced by adult moths mostly in the context of defense against bats, to detect  
72 and interfere with their predatory communication (Spangler 1986; Conner 1999; Minet and  
73 Surlykke 2003). However, larval acoustic emissions have scarcely been investigated and the  
74 behavioural context in which signals are produced has rarely been examined. Acoustic signals  
75 emitted by caterpillars can function as an enemy deterrent in *Antispila nysaefoliella*  
76 (Heliozelidae: Low 2008) and *Amorpha juglandis* (Sphingidae: Bura et al. 2011), or could

77 represent aposematic displays in the case of *Antherea polyphemus* larvae (Saturniidae: Brown  
78 et al. 2007). The use of vibrations (substrate-borne sounds) for territory defence has been  
79 documented for the tortricid moth *Sparganothis pilleriana* (Russ 1969), in drepanids *Drepana*  
80 *arcuata* (Yack et al. 2001), *Falcaria bilineata* (Bowen et al. 2008) and *Oreta rosea* (Scott et  
81 al. 2010) and in the gracillariidae *Caloptilia serotinella* (Fletcher et al. 2006).

82 When considering only “butterfly” acoustics, lycaenids *s.l.* (Lycaenidae and Riodinidae)  
83 represent one of the most studied groups. In lycaenids, larval emissions were documented  
84 almost one century ago (Farquharson et al. 1922), but their presence, role and mechanisms of  
85 production have not been clarified in many species. Most lycaenid species lack obvious larval  
86 stridulatory organs (Schurian and Fiedler 1991), although *Arhopala maditus* is an exception,  
87 as it possesses a stridulatory apparatus similar to those of pupae (Hill 1993), made of a series  
88 of spines rubbing against a continuous surface, which acts like a plectrum (Alvarez et al.  
89 2014). Nevertheless, in a survey of 130 butterfly species, DeVries (1990, 1991) revealed that  
90 19 species of Riodinidae and 30 species of Lycaenidae were able to produce air-borne sounds,  
91 typically as single pulses (i.e. call fundamental unit) or as pulse trains. These species were not  
92 necessarily closely related, neither did their geographical distributions overlap. However, all  
93 the species able to emit calls showed a certain degree of interaction with ants. Therefore,  
94 DeVries (1990, 1991) hypothesized that lycaenid and riodinid emissions may fulfil an  
95 important role for interacting with ants (“myrmecophily”), a relationship present in  
96 approximately 75% of the 6000 estimated species (DeVries 1990, 1991).

97 Lycaenid associations with ants may be mutualistic or parasitic and vary from facultative to  
98 strictly obligate. Myrmecophilous interactions can be nonspecific, especially if facultative, as  
99 the ants involved could belong to several species, or even subfamilies, or may be strictly  
100 species-specific. Immature butterfly stages could depend on ants for short periods or,

101 conversely, for the majority of their life cycle (for a detailed review about myrmecophily in  
102 lycaenids see Pierce et al. 2002).

103 Evidence of the use of lycaenid larval sounds in enhancing the interaction with ants is  
104 reported by Travassos and Pierce (2000), who found that the calls produced by pupae and  
105 caterpillars of *Jalmenus evagoras* are important in attracting *Iridomyrmex* host ants and in  
106 eliciting attendance behaviours. Other studies have highlighted the resemblance between ant  
107 stridulations and lycaenid calls (DeVries et al. 1993; Barbero and Casacci 2015), and  
108 documented how acoustic mimicry is employed by obligate parasitic *Maculinea* larvae to fool  
109 their host ant and live undisturbed within the host nests (Barbero et al. 2009 a, 2009b; Sala et  
110 al. 2014).

111 Sound production also occurs in non-myrmecophilous species, but these calls were  
112 documented as being emitted less frequently and appeared to be simpler than those of  
113 myrmecophilous species (Fiedler et al. 1995), reinforcing the hypothesis that acoustics of  
114 lycaenids may have a crucial role in their interactions with ants, while only playing a general  
115 defensive function in species that are not associated with ants.

116 The aim of this study was to describe and investigate the acoustic repertoire of 12 European  
117 (one of which, *C. marshalli*, was introduced) lycaenid larvae belonging to different  
118 subfamilies and representing several degrees of associations with ants (varying from no  
119 relationship to obligate parasitism). Specifically, we tested three hypotheses: (i) sounds are  
120 species-specific; (ii) myrmecophilous species are able to produce more complex acoustic  
121 emissions than species having no relationship with ants (iii) resemblance in acoustic patterns  
122 are explained by similarities in associations with ants rather than in phylogenetic distances  
123 between species.

## 124 **Materials and methods**

125 ***Species pool and sampling***

126 Larvae belonging to 12 species of lycaenids were field-collected from May 2012 to  
127 September 2014 at various sites across Northern Italy, with the exception of *Lycaena helle*  
128 (Poland: Table 1). When available, fully-developed caterpillars were collected, otherwise we  
129 gathered the food plants on which oviposition had occurred in order to rear the larvae in the  
130 laboratory.  
131 Specimens and their respective food plants were maintained in a rearing room within  
132 transparent boxes (30 × 20 × 20 cm<sup>3</sup>) covered with fine nets at a temperature of 18°C (night) :  
133 25°C (day) and at cycles of 14 h light/10 h dark. Following the experiments, caterpillars were  
134 released at their original location. Species were selected according to their degree of  
135 interaction with ants, with five degrees of association according to Fiedler (1991a, 1991b):  
136 namely no ant association (0 - myrmecoxenous), very few ant-associations reported, stable  
137 ant-associations only formed exceptionally (1 - weakly myrmecophilous), a varying  
138 proportion of larvae attended by ants (2 - moderately myrmecophilous), most if not all mature  
139 larvae associated with ants (3 - steadily myrmecophilous), larvae fully dependent on ants as  
140 commensals or parasites (4 - obligate myrmecophilous) (Table 1). All myrmecophilous  
141 species belonged to the Polyommatainae subfamily, while *Lycaena helle*, *L. dispar* and *L.*  
142 *phleas* were members of the subfamily Lycaeninae. The latter three species are not considered  
143 truly myrmecophilous, although they do possess morphological characteristics often  
144 associated with myrmecophily, such as specialised dendritic setae (Fiedler 1991a).

145 ***Sound recording***

146 We used a custom-made (Figure 1(a)) device that allows the recording of undisturbed  
147 (unstressed) late-instar caterpillars (three samples per species). The recording equipment  
148 consisted of a 12.5 × 8 × 2 cm<sup>3</sup> recording chamber with a moving-coil miniature microphone



149 (sensitivity: 2.5 mV/Pa/1.0 kHz) attached through the centre (sampling rate set to 44.10  
150 kHz). A second identical moving-coil microphone was used to record the ambient noise in  
151 anti-phase. A mixer and output amplifier (dynamic range: 5Hz to 40kHz; gain: 53dB)  
152 combined the signals from the two microphone preamplifiers. Overall, the frequency ranged  
153 from 20 Hz to 20 kHz and the gain was approximately 83 dB. The equipment was powered by  
154 a 12V gel cell battery. The recording chamber (Figure 1 (b)) and the microphone were located  
155 inside an anechoic chamber to further reduce ambient noise and interference. Late instar  
156 caterpillars were individually placed on the microphone surface within the recording chamber  
157 and recorded in the morning at room temperature (23-25°C). Samples were recorded for 20-  
158 minute periods, starting at 5 minutes after the introduction of the caterpillar in the recording  
159 chamber.

160 Segments containing acoustic recordings were carefully inspected, checked for clipping and  
161 digitally saved in WAV format (16-bit amplitude resolution) using Audacity v. 1.2.4  
162 (<http://audacity.sourceforge.net/>). The temporal and spectral features of the signals were  
163 measured using Praat v. 5.3.53 (<http://www.fon.hum.uva.nl/praat/>).

#### 164 *Call comparisons and statistical analysis*

165 We analysed recordings of three individuals per species. Firstly, we assessed variations in call  
166 complexity investigating whether the pulse-interval patterns (classified by Sandberg (2011) as  
167 monophasic, varied beat-interval, diphasic and grouped, from the simplest to more complex  
168 patterns) and the pulse spectrographic characteristics varied within and across species (from  
169 pure tones to more complex frequency patterns). We then randomly selected two trains of five  
170 pulses for each individual (Figure 1 (c), Figure 2) and measured, for each pulse a total of 21  
171 temporal and spectral parameters (Supplementary Information Table S1). We then computed  
172 a pairwise correlation analysis on the 21 acoustic parameters (Spearman-Rank-Correlation;

173 SPSS v22). From a pair of parameters with  $r_s > 0.85$ , only one was selected for analysis. This  
174 method yielded 12 acoustic variables (Figure 3, Table S2, S3). Six variables were  
175 automatically extracted using a script: the first and third quartiles of the energy spectrum ( $Q_{25}$   
176 and  $Q_{75}$ , Hz; 25% and 75% of the call energy); the relation of the frequency peak energy to  
177 the call total energy expressed as a percentage ( $\%E_{F_{peak}}$ ); the mean intensity of the entire call  
178 represented by the root-mean-square signal level (RMS, dB). Two temporal variables were  
179 also measured from the oscillogram: the pulse duration ( $\Delta_t$ , s) and the pulse rate (PR,  $s^{-1}$ ;  
180 calculated as  $1/t_{start(x)} - t_{start(x+1)}$ ).

181 The other six variables were manually estimated, for each pulse, by inspection of power  
182 spectra: the frequency of the first and second peak amplitudes ( $F_1$ ,  $F_2$ , Hz) and the intensity of  
183 the first three peak amplitudes ( $I_1$ ,  $I_2$ ,  $I_3$ , dB – see Figure 1 (d)) In addition we manually  
184 estimated the centre of gravity (CG, Hz; an average measure for how high the frequencies in a  
185 spectrum are).

186 To account for individual differences in sound production, we assessed the variation in each  
187 call parameter by using Generalised Linear Models (GLM) in which lycaenid species and  
188 myrmecophily categories were used as fixed factor and the “individual” as random factor.  
189 Post hoc tests with Bonferroni correction reported pairwise differences between categories  
190 (Table S4, S5). Analyses were performed using SPSS v.22.

191 To test whether sounds differed between groups, we calculated the pairwise normalized  
192 Euclidean distances over sound parameters and used the ANalysis Of SIMilarity (ANOSIM)  
193 implemented in Primer v. 6.1.12 (Primer-E Ltd.) to assess differences between species,  
194 subfamilies, and myrmecophilous levels. Groupings were visualised through nonparametric  
195 multi-dimensional scaling (nMDS) using single sound pulses (Figure 4). Similarity  
196 percentages (SIMPER) were used to calculate the individual contribution of each sound

197 parameter to the differences between myrmecophilous categories and their contribution to the  
198 similarity between species belonging to the same degree of lycaenid-ant association.

## 199 **Results**

200 All 12 species investigated in our study produced calls, i.e. signals composed of a variable  
201 number of pulses (Figure 2). We provide the first evidence of call production for *Cacyreus*  
202 *marshalli*, *Lycaena helle*, *L. dispar*, *Cupido argiades*, *Lycaeides argyrognomon*, *Scolitantides*  
203 *orion*, and *Plebejus argus*, while we confirm this ability in *Maculinea alcon*, *Lycaena phleas*,  
204 *Polyommatus bellargus*, *Polyommatus coridon* and *Polyommatus icarus* (DeVries 1991). Call  
205 complexity (i.e. different patterns in pulse/interval succession and pulse spectrum structure)  
206 was investigated across the various levels of association, from non-myrmecophilous to  
207 myrmecophilous species, but no differences were detected. All larvae emit monophasic  
208 signals (constant interval between regular pulses, *sensu* Sandberg 2011) repeating pulses that  
209 are stable in tone, duration, and pulse rate. Pulses consist of a short, repeated tone composed  
210 of a fundamental frequency followed by a variable number of harmonics (Figure 2) and are  
211 generally repeated in trains, but may be rarely delivered as isolated signals.

212 Overall, we did not find any differences in emission complexity (call and pulse structure)  
213 between species. We observed that pulses were characterized by the same spectrographic  
214 template (i.e. at least three frequency components with a harmonic structure in all our  
215 samples). Inter-peak values could occur between principal frequencies, and periodicity was  
216 obvious in all cases. Pulses exhibit a broadband frequency extending up to around 7 kHz, but  
217 most of the energy in the emissions was distributed below 3 kHz (Figure 2). Average  
218 measurements for the 12 sound parameters of each species are reported in Figure 3 (mean  
219 values are listed in Table S2).

## 220 ***Call comparisons: univariate analysis***

221 Sound parameters varied significantly between species (Figure 3), while no significant  
222 differences were observed between calls produced by specimens belonging to the same  
223 species (for each model  $P > 0.05$ ). Post hoc comparisons between species showed significant  
224 differences for most of the investigated parameters (Figure 3, Table S4). Differences were less  
225 pronounced between species belonging to the same myrmecophilous category (Table S5). No  
226 significant differences were detected in frequency components ( $F_1$ ,  $F_2$ ), intensities ( $I_2$ ,  $I_3$ ), and  
227 centre of gravity (CG) between *Licaena helle* and *L. dispar*, or in pulse duration and centre of  
228 gravity between *Polyommatus bellargus* and *P. icarus*. The investigated parameters did not  
229 show unequivocal trends on the basis of species taxonomy. Nevertheless, when excluding  
230 *Cacyreus marshalli*, given the different biogeography of this species, data showed patterns of  
231 variation related to different degree of interaction with ants (Figure 3). Species that were  
232 strictly associated with ants produced longer pulses ( $\Delta_t$ ), and the interval between the pulses  
233 tended to be shorter (PR). The parameters investigated on the pulse spectrum showed that  
234 frequency ( $F_1$ ,  $F_2$ ,  $Q_{25}$ ,  $Q_{75}$ , CG) were generally higher in species that were not associated  
235 with ants. Conversely, the percentage energy at the peak frequency ( $EF_{Peak}$ ) appeared to  
236 increase in myrmecophilous species, such as measures of signal intensity ( $I_1$ ,  $I_2$ ,  $I_3$  and RMS).  
237 *Maculinea alcon*, an obligate parasite of *Myrmica* ants, generally showed the extreme values  
238 for all analysed parameters and it significantly differs from other species for the first  
239 frequency component, the first and third frequency quartiles, the centre of gravity and the  
240 intensity of the second peak amplitude.

241

#### 242 ***Call comparisons: multivariate analysis***

243 Nonparametric multi-dimensional scaling (nMDS, Figure 4), carried out on all sound  
244 parameters recorded from the 12 lycaenid species, showed group separations, and the  
245 ANalysis Of SIMilarity (ANOSIM) statistically discriminated the pulses on the factor

246 “species” (ANOSIM: Global  $R=0.768$ ;  $P=0.001$ ). Moreover, all pairwise comparisons  
247 resulted as being significant (ANOSIM:  $0.249 < R < 1$ ;  $P=0.001$ ).

248 In the nMDS graph (Figure 4) individual call pulses produced by species belonging to the  
249 subfamily Lycaeninae (*L. dispar*, *L. helle*, *L. phleas*) and Polyommatinae are grouped  
250 separately (ANOSIM:  $R=0.185$ ;  $P=0.001$ ). The SIMPER analysis shows that the similarity of  
251 acoustic emissions of *Lycaena* species is mainly explained by the length of the pulses, and  
252 frequencies ( $F_1$  and  $F_2$ ) and intensity ( $I_2$  and  $I_3$ ) of the first three harmonics. Pulses were also  
253 distinguishable on the basis of the level of myrmecophily (following the categorization  
254 according to Fiedler 1991a,b) (ANOSIM: Global  $R=0.356$ ;  $P=0.001$ ) but, when considering  
255 the pairwise similarity tests, sounds produced by non-myrmecophilous species (level=0) did  
256 not differ from *C. argiades* calls (level=2; ANOSIM:  $R=0.019$ ;  $P=0.341$ ) or *P. icarus*  
257 (level=2/3, ANOSIM:  $R=-0.123$ ;  $P=0.998$ ). We also found no significant differences between  
258 the acoustic signals emitted by the latter species and those produced by species classified as  
259 “steadily myrmecophilous” (level=3, ANOSIM:  $R=-0.108$ ;  $P=0.982$ ). If we remove the pulses  
260 recorded from *C. marshalli* from the analysis, all the previous pairwise comparisons differ  
261 significantly, apart from calls emitted by *P. icarus* (level 2/3), which still cluster together with  
262 those produced by species having myrmecophilous level 3 (level 2/3 vs. 3; i.e. *P. icarus* vs. *L.*  
263 *argyrognomon*, *P. coridon*, *P. bellargus*, *S. orion* - ANOSIM:  $R=-0.108$ ;  $P=0.988$ ). The non-  
264 myrmecophilous species (*Lycaena* spp.) and *Maculineaalcon*, which have the highest degree  
265 of specialisation on ants, were found to be the most distant groups (SIMPER<sub>average square distance</sub> =  
266 61.51). Four frequency components account for more than 50% of call dissimilarity (i.e. the  
267 first frequency component (15.9%), the third (12.3%) and first quartile (12.1%) of frequency  
268 and the centre of gravity (11.4%)).

269

270 **Discussion**

271 This study describes and measures similarities between the calls produced by caterpillars of  
272 12 lycaenid species, which were selected to represent different subfamilies and degrees of  
273 association with ants. All the species investigated produced acoustic signals, providing the  
274 first evidence of this ability for seven species, and supporting the hypothesis that acoustic  
275 communication is widespread among lycaenids, independently from phylogeny, biogeography  
276 and level of association with ants (Fiedler 1991a; Pierce et al. 2002).

277 Our study showed that lycaenid calls are distinct, species-specific signals. Pulses produced by  
278 different species were significantly distinguishable, while conspecific individuals shared  
279 similar call features.

280 Patterns of sound species-specificity have been previously observed in other insect taxa, and  
281 several studies have reported that it is possible to discriminate even “cryptic” species on the  
282 basis of their acoustic emissions (e.g. Hymenoptera: Ferreira et al. 2010; Carlos et al. 2014;  
283 Heteroptera: Lampson et al. 2013; Orthoptera: Chesmore 2004). However, studies measuring  
284 call variation and similarities in lycaenid species are lacking. Recent studies have focused on  
285 single species, sometime comparing them to specific host ants (e.g. Travassos and Pierce  
286 2000, Barbero et al. 2009 a, b, Barbero et al. 2012), while DeVries (1991), in his seminal  
287 study, only described the individual calls made by 30 lycaenid species.

288 Regarding the five species formerly investigated by DeVries (1991), we obtained similar  
289 mean values of temporal parameters, but we recorded broader frequency ranges in the case of  
290 *Polyommatus coridon*, *P. bellargus* and *P. icarus*. These variations between the two studies  
291 may be due to both technical limits of the recording equipment used 25 years ago and to the  
292 method performed for inducing call emission. Lycaenid sounds have previously been  
293 collected by stressing larvae with tweezers (e.g. DeVries 1991, Travassos and Pierce 2000),

294 while our recording device allowed us to collect calls without harming the caterpillars, which  
295 could therefore behave more naturally (Barbero et al. 2009b).

296 All caterpillar specimens emitted calls, despite being unstressed and irrespectively of whether  
297 they were myrmecophilous or not.

298 Given that sound production has associated energetic costs (Prestwich 1994), these emissions  
299 should provide advantages and increase fitness in both myrmecophilous and myrmecoxenous  
300 caterpillars. Thus for those species not interacting with ants (e.g. *Lycaena* spp.), acoustic  
301 emissions could be useful to signal and repel natural enemies, as observed in other  
302 Lepidoptera (Bura et al. 2009, 2011), but could also be employed in intraspecific contexts.  
303 For instance, in the case of a high density of *Cacyreus marshalli* caterpillars sharing the same  
304 larval host plant, sounds may be employed as territorial signals to communicate the presence  
305 of conspecifics on the food/shelter resource, which has been observed for some moth larvae  
306 (see e.g. Yack et al. 2001).

307 Myrmecophilous associations better explain the pattern of acoustic similarity revealed by the  
308 present study than taxonomic relationships. For instance, within the Polyommatae  
309 subfamily, congeneric species emit rather dissimilar signals (e.g. *Polyommatus icarus* calls  
310 are more similar to those of *Lycaeides argyrognomon* than to those produced by *Polyommatus*  
311 *coridon*, Figure 4). If we consider only European species (thus excluding *C. marshalli*, an  
312 invasive South African species in Europe) calls can be significantly grouped according to  
313 their degree of myrmecophily, as displayed by the multi-dimensional scaling plot (Figure 4).  
314 The most diverse calls are produced by *Lycaena* spp. and *M. alcon*, representing, respectively,  
315 the lowest (not myrmecophilous) and the highest (obligate social parasite) degrees of  
316 myrmecophily according to Fielder's classification.

317 Some of the sound parameters clearly define different myrmecophilous and myrmecoxenous  
318 groups and for some of them, an evident trend reflecting the degree of association with ants

319 exists. This is particularly true for temporal characteristics, such as pulse duration and pulse  
320 rate, with species that are less associated with ants producing shorter and closer pulses  
321 compared to those that are highly dependent on ants. At the same time, SIMPER analysis  
322 showed that four frequency parameters contributed to more than 50% of the dissimilarity in  
323 acoustic emission between myrmecophilous and myrmecoxenous species, suggesting that call  
324 effectiveness probably does not rely on a higher sound complexity, but more likely in  
325 possessing an appropriate frequency spectrum to better stimulate attending ants.

326 Indeed, in contrast to previous statements (Fiedler et al. 1995, Travassos and Pierce 2000), we  
327 did not observe differences in signal complexity between ant-associated and non-  
328 myrmecophilous larvae for the studied lycaenids. Calls appeared to be monotonic, and all  
329 species displayed a distinctive spectral pattern constituted by a fundamental frequency and a  
330 distinctive number of harmonic frequencies (Figure 2). This pattern has also been reported for  
331 many other insects (Hung and Prestwich 2004; Gogala and Trilar 2007; Cator et al. 2009), but  
332 is described here for lycaenid calls for the first time.

333 The frequencies observed during this study in lycaenid calls were similar to those emitted by  
334 ants potentially associated with them (e.g. *gen. Myrmica*, Casacci et al. 2013) and previous  
335 bioassays have demonstrated the ability of the call produced by *Maculinea* spp., obligate  
336 parasites, to elicit a behavioural response in host ant workers (Barbero et al. 2009 a, Sala et al.  
337 2014).

338 Calls of species strictly associated with ants (e.g. *P. coridon*, *P. argus*, *M.alcon*) are  
339 characterized by lower fundamental frequency, which means a higher number of harmonics  
340 within the frequency range of the ants, potentially increasing the chance to stimulate ant  
341 receptors.

342 Even though information on central auditory systems of insects is scarce, it has been showed  
343 that neurons have a remarkable selectivity to acoustic signal properties such as frequencies



344 (Gerhardt and Huber 2002). According to the “matched-filter hypothesis”, receptors are  
345 hypothesized to match “the physical properties of the sender's signal” (Wehner 1987) and the  
346 receiver would benefit from being selectively tuned to particular signal features. This has  
347 been observed in acoustic behaviour of orthopterans, where different neurons are tuned into  
348 unique frequencies involved in sexual communication and directional cues for localization  
349 (Kostarakos et al. 2008), but could also occur in ants, given the increasing evidence of the  
350 important role played by acoustic signals in ant colony communication (Settele et al. 2011) .

351

### 352 *Conclusion*

353 The results reported here, given the widespread occurrence of call production in lycaenids,  
354 and considering the biological context in which they are produced, suggest that calls have  
355 evolved from repellent or territorial signals towards lure calls for attracting specific ant taxa.  
356 Nevertheless, modalities of signal production, transmission and reception remain largely  
357 unknown for most lycaenid species (including those studied here). Direct comparisons  
358 between caterpillar calls and host ant stridulations, coupled with playback bioassays, are  
359 required in order to reach any further conclusions. Behavioural experiments for testing ant  
360 reactions to lycaenid acoustic stimuli will provide clear-cut insights into the biological  
361 meaning of acoustic communication in this complex inter-dependent system.

362

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486

487 **Tables**

488 Table 1: Species investigated (N=36) and related collecting locations. Country, Locality,  
 489 UTM coordinates and altitude, as along with level of association with ants, according to  
 490 Fiedler's classification (1991), are reported. Degrees of myrmecophily: 0= myrmecoxenous;  
 491 0/1= weakly myrmecophilous; 2= moderately myrmecophilous; 3= steadily myrmecophilous;  
 492 4= obligate myrmecophilous (2/3 and 3/4 are intermediate categories). <sup>a</sup>In the extensive  
 493 literature on this locally endangered species and in recent textbooks, no mention of  
 494 myrmecophilous interaction is given. Only two old records of ant-associations exist. Like all  
 495 other European *Lycaena* species for which appropriate information is available, *L. dispar* is  
 496 probably not truly myrmecophilous (Fiedler 1991).

| Species                       | Country | Locality           | UTM coordinates       | Altitude<br>(m) | Level of<br>myrmecophily<br>(Fiedler 1991) |
|-------------------------------|---------|--------------------|-----------------------|-----------------|--|
| <i>Cacyreus marshalli</i>     | Italy   | Torino             | N 32 T 396631 4991600 | 234             | 0  |
| <i>Lycaena phleas</i>         | Italy   | Venaria            | N 32 T 392350 4998321 | 258             | 0  |
| <i>Lycaena helle</i>          | Poland  | Ogrodniczki        | N 34 U 651141 5895299 | 132             | 0  |
| <i>Lycaena dispar</i>         | Italy   | Trino Vercellese   | N 32 T 444750 5004777 | 131             | 0 <sup>a</sup>                             |
| <i>Cupido argiades</i>        | Italy   | Venaria            | N 32 T 392350 4998321 | 258             | 2  |
| <i>Polyommatus icarus</i>     | Italy   | Castelnuovo Scrvia | N 32 T 490624 4980821 | 92              | 2/3  |
| <i>Lycaeides argyrognomon</i> | Italy   | Venaria            | N 32 T 392350 4998321 | 258             | 3  |
| <i>Polyommatus bellargus</i>  | Italy   | Venaria            | N 32 T 392350 4998321 | 258             | 3  |
| <i>Polyommatus coridon</i>    | Italy   | Terme di Valdieri  | N 32 T 362013 4896424 | 1373            | 3  |
| <i>Scolitantides orion</i>    | Italy   | Padova             | N 32 T 725123 5032127 | 25              | 3  |
| <i>Plebejus argus</i>         | Italy   | Terme di Valdieri  | N 32 T 362013 4896424 | 1373            | 3/4  |
| <i>Maculineaalcon</i>         | Italy   | Caselette          | N 32 T 380608 4996043 | 391             | 4  |

497

498



499 **Figure captions**

500 Figure 1: Recording and analysis of acoustic parameters of lycaenid larval calls. (a)  
501 Customised recording device. (b) Detailed view of the recording chamber. (c) Oscillogram  
502 and spectrogram (window size, 1024). Lycaenid calls all share the same basic acoustic  
503 structure: pulses are generally repeated in trains and usually consist of at least three frequency  
504 components with a harmonic structure, the first of which is the fundamental frequency. (d)  
505 Power spectrum. See text for a description of measured parameters.

506  
507 Figure 2: Example waveforms (upper traces) and spectrograms (lower traces) of sounds  
508 emitted by the larvae of the 12 lycaenid species. Spectrograms were generated in Praat using a  
509 Gaussian window shape, window length from 0.02 to 0.008 s, number of time steps = 1000,  
510 number of frequency steps = 500, dynamic range = 50 dB.

511  
512 Figure 3: Boxplots show median, quartile, maximum and minimum values; outliers are open  
513 circles. Differences between species were tested for each parameter: 1<sup>st</sup> frequency component  
514 ( $F_1 - F_{360,11}=47.089$ ;  $P<0.001$ ); 2<sup>nd</sup> frequency component ( $F_2 - F_{360,11}= 18.734$ ;  $P<0.001$ ); 1<sup>st</sup>  
515 frequency quartile ( $Q_{25} - F_{360,11}=19.749$ ;  $P<0.001$ ); 3<sup>rd</sup> frequency quartile ( $Q_{75} -$   
516  $F_{360,11}=12.818$ ;  $P<0.001$ ); Centre of gravity ( $CG - F_{360,11}=8.798$ ;  $P<0.001$ ); percentage energy  
517 at the peak frequency ( $\%E_{F_{peak}} - F_{360,11}=10.266$ ;  $P<0.001$ ); pulse rate ( $PR - F_{360,11}=30.435$  ;  
518  $P<0.001$ ); pulse duration ( $\Delta_t - F_{360,11}=62.345$ ;  $P<0.001$ ); intensity of the 1<sup>st</sup> peak amplitude ( $I_1$   
519  $- F_{360,11}=3.953$ ;  $P= 0.003$ ); intensity of the 2<sup>nd</sup> peak amplitude ( $I_2 - F_{360,11}=14.386$ ;  $P<0.001$ );  
520 intensity of the 3<sup>rd</sup> peak amplitude ( $I_3 - F_{360,11}=11.381$ ;  $P<0.001$ ); Root-Mean-Square signal  
521 level ( $RMS - F_{360,11}=14.134$ ;  $P<0.001$ ).

522 The letters above each column indicate significance in post hoc tests (Table S4). The same  
523 letter indicates no significant difference between species in pairwise Bonferroni tests  
524 ( $P>0.05$ ).

525

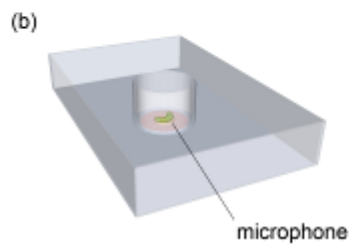
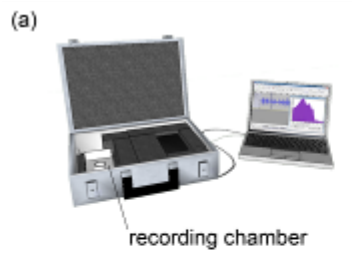
526 Figure 4: 2-dimensional nMDS ordination of the normalised Euclidean distances over all  
527 single parameters analysed on individual pulses of the 12 lycaenid species. Each species  
528 centroid reports the degree of myrmecophilous interaction, as categorized by Fiedler (1991).

529

530

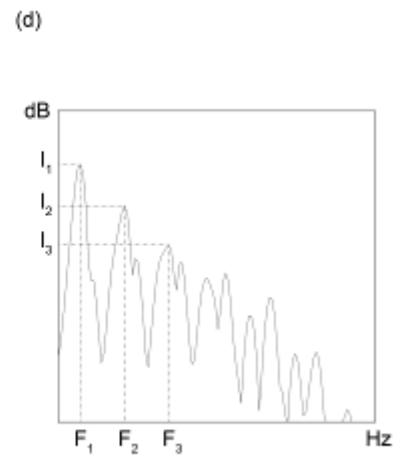
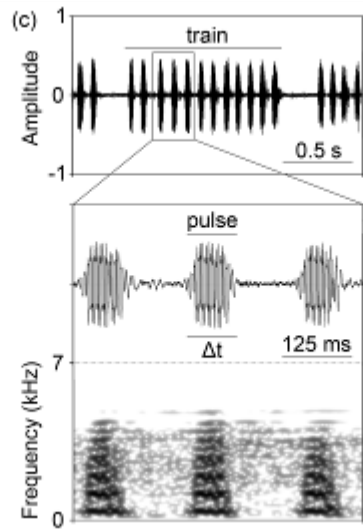
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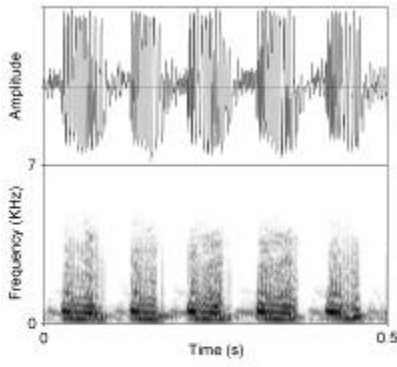


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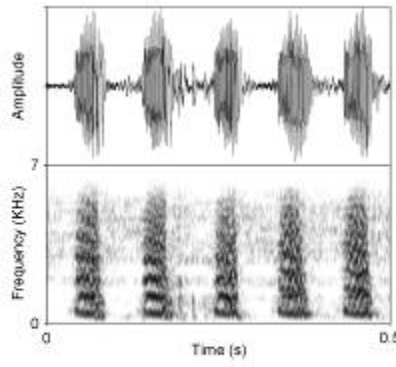
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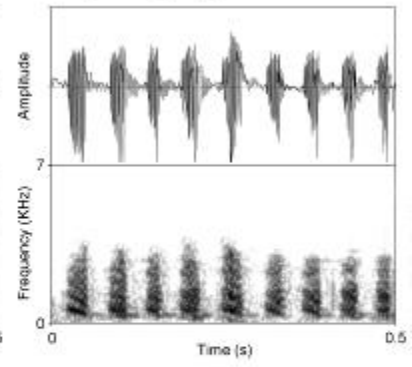
*Maculinea alcon*



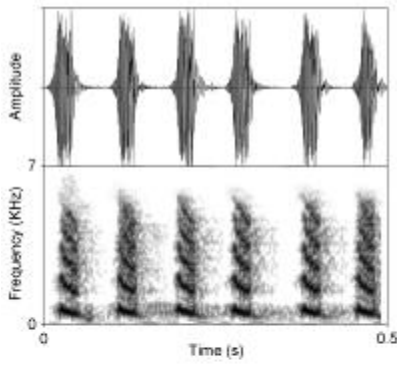
*Plebejus argus*



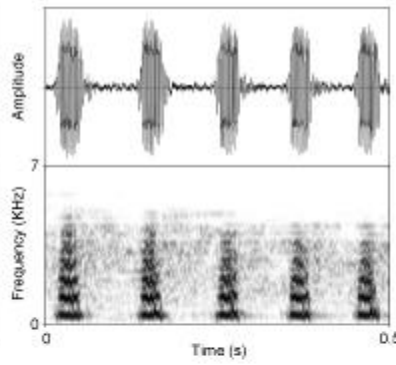
*Plebejus argyrognomon*



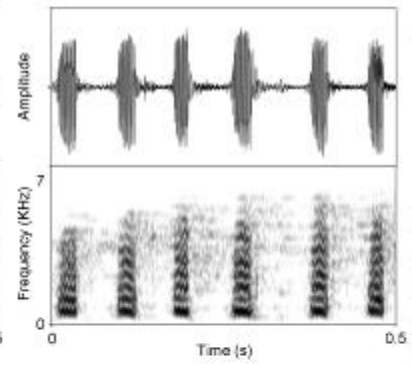
*Scolitantides orion*



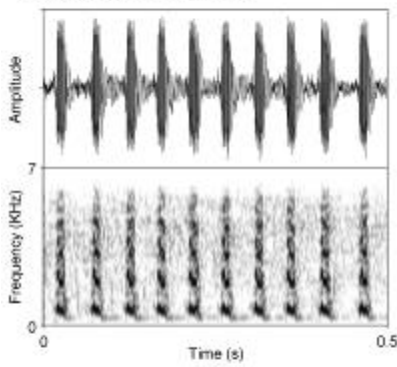
*Polyommatus coridon*



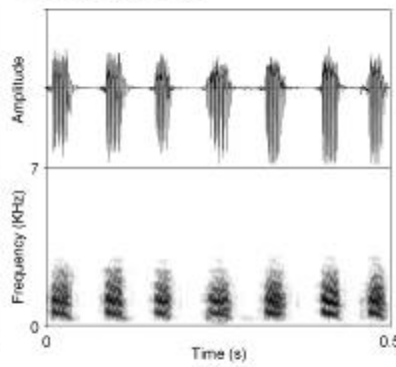
*Polyommatus bellargus*



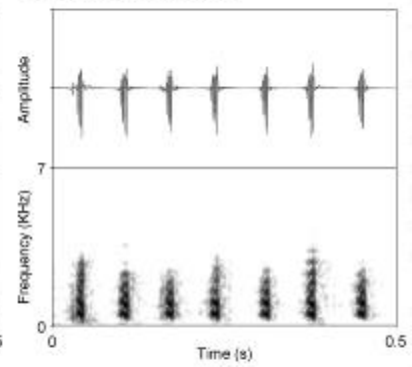
*Polyommatus icarus*



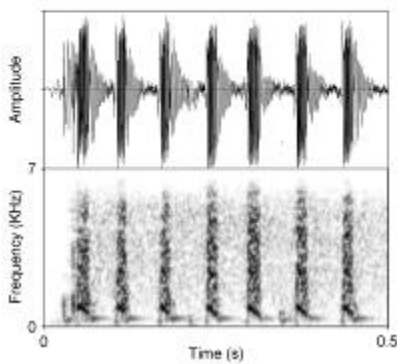
*Cupido argiades*



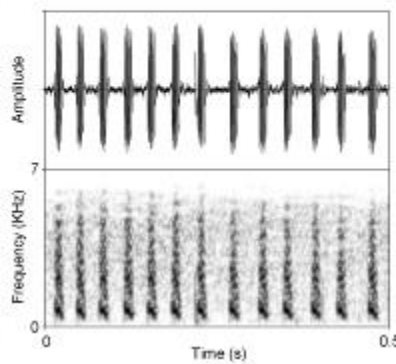
*Cacyreus marshalli*



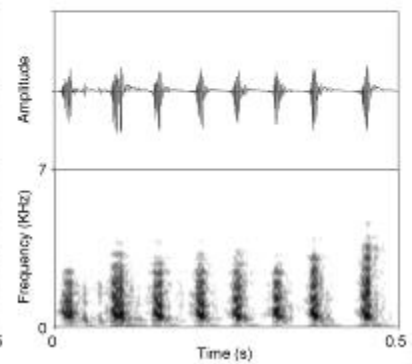
*Lycaena helle*



*Lycaena dispar*

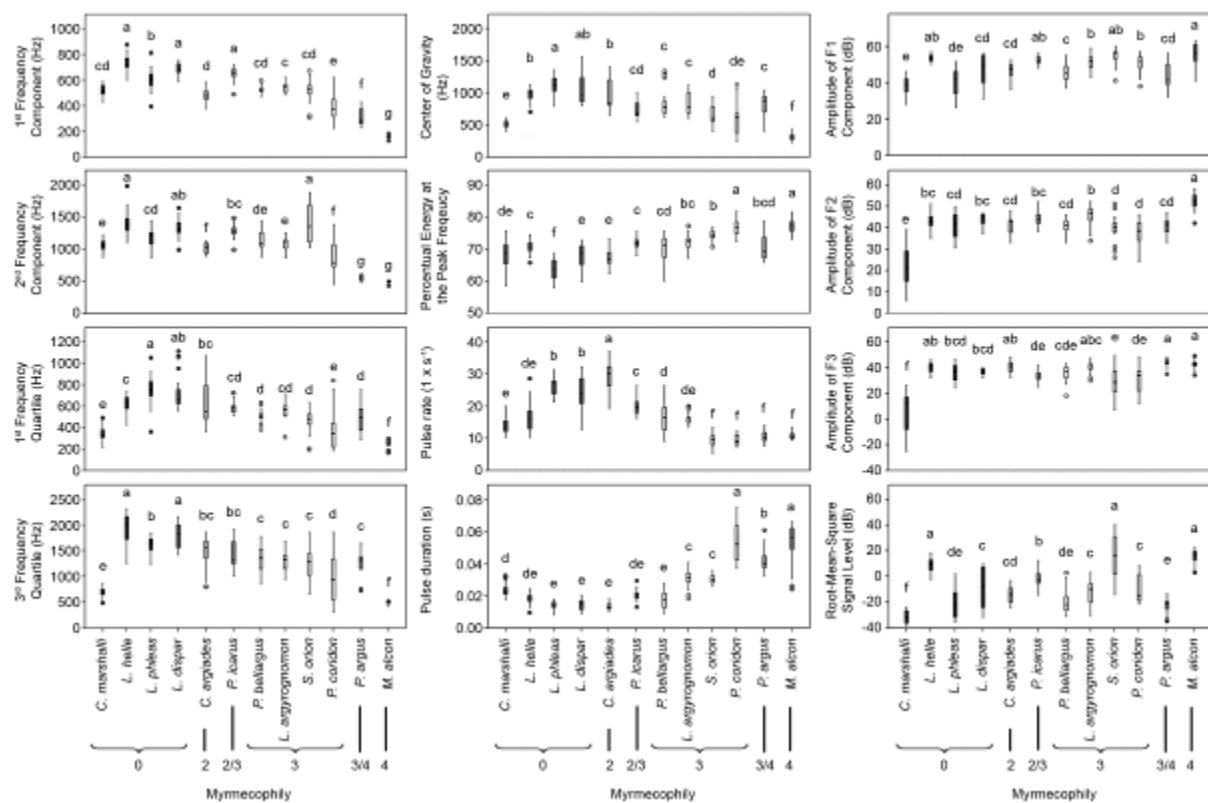


*Lycaena phleas*



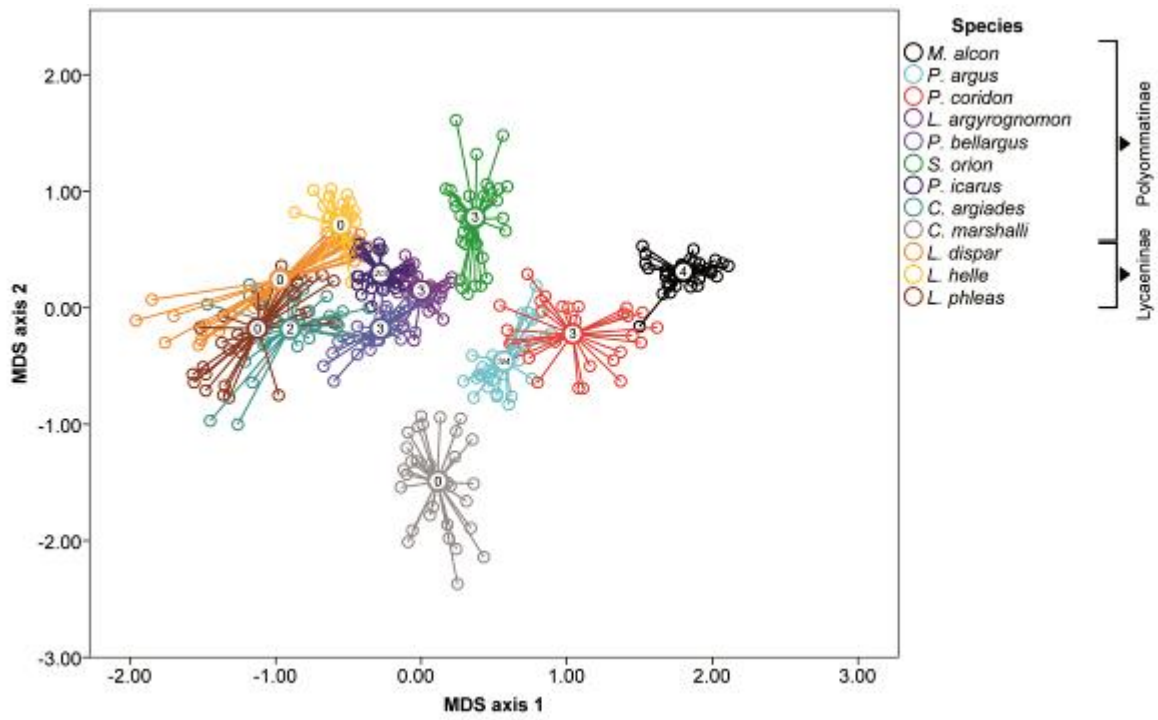
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541 Table S1. List of the 21 acoustic parameters measured on the pulses (i.e. call fundament unit)  
 542 of each species

| <b>Acoustic parameters</b> |   | <b>Unit</b>                     | <b>Measurement</b> |
|----------------------------|---|---------------------------------|--------------------|
| Q <sub>25</sub>            | Lower quartile of the energy spectrum   | Hz                              | Automatic          |
| Q <sub>50</sub>            | Central quartile of the energy spectrum                                       | Hz                              | Automatic          |
| Q <sub>75</sub>            | Higher quartile of the energy spectrum  | Hz                              | Automatic          |
| SD <sub>Q50</sub>          | Frequency standard deviation  | Hz                              | Automatic          |
| F <sub>peak</sub>          | Frequency peak  | Hz                              | Automatic          |
| E <sub>Fpeak</sub>         | Energy of the frequency peak  | Pa <sup>2</sup> s <sup>-1</sup> | Automatic          |
| %E <sub>Fpeak</sub>        | Relation of the frequency peak energy to the call total energy                | %                               | Automatic          |
| A <sub>a</sub>             | Mean amplitude  | dB                              | Automatic          |
| P                          | Power   | dB <sup>2</sup>                 | Automatic          |
| E                          | Energy  | Pa <sup>2</sup> s <sup>-1</sup> | Automatic          |
| I                          | Intensity value   | dB                              | Automatic          |
| RMS                        | Root- mean-square signal level  | dB                              | Automatic          |
| Δ <sub>t</sub>             | Duration of the pulse   | s                               | Automatic          |
| PR                         | Pulse Rate, calculated as 1/(t <sub>start(x)</sub> -t <sub>start(x+1)</sub> ) | s <sup>-1</sup>                 | Automatic          |
| F <sub>1</sub>             | Frequency of the first peak amplitude of the power spectrum                   | Hz                              | Manual             |
| F <sub>2</sub>             | Frequency of the second peak amplitude of the power spectrum                  | Hz                              | Manual             |
| F <sub>3</sub>             | Frequency of the third peak amplitude of the power spectrum                   | Hz                              | Manual             |
| I <sub>1</sub>             | Intensity of the first peak amplitude of the power spectrum                   | Hz                              | Manual             |
| I <sub>2</sub>             | Intensity of the second peak amplitude of the power spectrum                  | Hz                              | Manual             |
| I <sub>3</sub>             | Intensity of the third peak amplitude of the power spectrum                   | Hz                              | Manual             |
| CG                         | Centre of gravity   | Hz                              | Manual             |

543

544 Table S2. Mean values ( $\pm$  SD) of the 12 temporal and spectral parameters, collected for the 12  
 545 butterfly species

|                        | $\Delta_t$ (s) | PR ( $s^{-1}$ ) | F <sub>1</sub> (Hz) | I <sub>1</sub> (dB) | F <sub>2</sub> (Hz) | I <sub>2</sub> (dB) | I <sub>3</sub> (dB) | CG (Hz)          | RMS (dB)    | Q <sub>2s</sub> (Hz) | Q <sub>7s</sub> (Hz) | %E <sub>Fpeak</sub> (%) |
|------------------------|----------------|-----------------|---------------------|---------------------|---------------------|---------------------|---------------------|------------------|-------------|----------------------|----------------------|-------------------------|
| <i>C. marshalli</i>    | 0.023±0.003    | 14.260±2.693    | 519.846±45.536      | 38.115±4.982        | 1057.615±92.366     | 23.461±8.276        | 4.515±5.428         | 517.615±51.482   | 0.078±0.046 | 333.657±67.024       | 686.377±98.565       | 68.046±4.041            |
| <i>L. dispar</i>       | 0.014±0.003    | 24.297±4.753    | 689.033±47.347      | 46.553±8.760        | 1330.433±136.462    | 43.700±2.854        | 35.933±4.471        | 1061.6±240.276   | 0.309±0.164 | 716.689±147.005      | 1787.795±233.564     | 67.66±3.993             |
| <i>L. helle</i>        | 0.018±0.003    | 16.407±4.326    | 729.566±62.186      | 53.433±1.977        | 1402.266±161.318    | 42.866±3.501        | 40.666±3.950        | 955.5±159.160    | 0.489±0.061 | 619.896±84.788       | 1917.821±297.232     | 70.656±1.993            |
| <i>L. phleas</i>       | 0.013±0.002    | 25.930±2.516    | 608.769±70.848      | 41.096±7.129        | 1199.153±123.648    | 40.95±6.482         | 35.950±7.561        | 1092.846±139.122 | 0.202±0.114 | 748.048±137.203      | 1586.547±168.301     | 64.212±3.108            |
| <i>C. argiades</i>     | 0.013±0.001    | 29.573±4.011    | 476.576±51.304      | 44.261±9.773        | 1004.192±79.138     | 40.357±3.819        | 39.673±3.702        | 921.5±242.37     | 0.240±0.060 | 640.87±22.323        | 1491.225±277.280     | 66.918±2.206            |
| <i>P. icarus</i>       | 0.020±0.002    | 19.999±2.498    | 653.076±41.904      | 52.346±2.152        | 1290.730±84.133     | 44.500±3.140        | 34.115±3.902        | 723.307±123.234  | 0.377±0.063 | 585.498±52.645       | 1400.419±261.195     | 71.967±1.663            |
| <i>S. orion</i>        | 0.030±0.002    | 9.850±1.860     | 507.73±91.397       | 53.500±5.770        | 1366.115±281.314    | 37.576±7.895        | 30±13.514           | 657.884±151.996  | 0.550±0.169 | 467.16±108.901       | 1322.038±304.937     | 74.299±1.474            |
| <i>P. bellargus</i>    | 0.018±0.005    | 16.508±4.681    | 527.961±25.246      | 46.307±5.416        | 1164.384±158.214    | 40.538±3.088        | 35.384±8.913        | 824.038±221.363  | 0.195±0.093 | 509.727±52.009       | 1379.906±240.306     | 70.368±3.546            |
| <i>P. argyrognomon</i> | 0.031±0.004    | 15.753±1.370    | 531.846±31.703      | 50.526±3.951        | 1064.500±88.886     | 45.719±4.410        | 39.619±5.112        | 838.73±176.436   | 0.260±0.082 | 563.715±80.509       | 1276.871±194.715     | 72.339±2.422            |
| <i>P. coridon</i>      | 0.053±0.011    | 9.254±1.601     | 374.192±96.326      | 50.307±4.654        | 784.153±228.309     | 38.269±4.065        | 31.500±8.936        | 589.461±251.978  | 0.299±0.111 | 390.621±155.782      | 1073.975±412.700     | 76.478±2.453            |
| <i>P. argus</i>        | 0.041±0.006    | 10.524±1.836    | 317.961±60.027      | 44.692±6.442        | 552.500±48.228      | 40.461±3.679        | 43.692±3.495        | 798.846±201.968  | 0.172±0.072 | 485.001±119.232      | 1211.163±267.930     | 70.823±3.722            |
| <i>M. alcon</i>        | 0.054±0.009    | 10.729±1.044    | 160.961±12.327      | 56.576±5.981        | 491.307±31.820      | 51.653±3.497        | 41.961±3.304        | 303.692±41.498   | 0.550±0.059 | 189.626±49.540       | 501.304±31.893       | 77.293±2.247            |

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547 Table S3. Mean values ( $\pm$  SD) of temporal and spectral parameters of each myrmecophilous  
 548 category (0= myrmecoxenous; 2= moderately myrmecophilous; 3= steadily myrmecophilous; 4=  
 549 obliged myrmecophilous)

| Myr-<br>mecophi-<br>lous<br>category | PR (s <sup>-1</sup> ) | F <sub>1</sub> (Hz)   | I <sub>1</sub> (dB) | F <sub>2</sub> (Hz)    | I <sub>2</sub> (dB) | I <sub>3</sub> (dB)  | CG (Hz)                | $\Delta_t$ (s)    | RMS (dB)          | Q <sub>25</sub> (Hz)   | Q <sub>75</sub> (Hz)    | %E <sub>Fpeak</sub> (%) |
|--------------------------------------|-----------------------|-----------------------|---------------------|------------------------|---------------------|----------------------|------------------------|-------------------|-------------------|------------------------|-------------------------|-------------------------|
| 0                                    | 20.233 $\pm$ 6.183    | 641.982 $\pm$ 9.8069  | 45.17 $\pm$ 8.522   | 1255.866 $\pm$ 185.16  | 38.14 $\pm$ 9.845   | 29.911 $\pm$ 6.627   | 914.151 $\pm$ 2.78.04  | 0.017 $\pm$ 0.005 | 0.279 $\pm$ 0.185 | 609.124 $\pm$ 1.95.522 | 1520.219 $\pm$ 5.21.411 | 67.752 $\pm$ 4.036      |
| 2                                    | 29.573 $\pm$ 4.011    | 476.576 $\pm$ 5.1.304 | 44.261 $\pm$ 9.773  | 1004.192 $\pm$ 79.138  | 40.357 $\pm$ 3.819  | 39.673 $\pm$ 3.702   | 921.5 $\pm$ 242.370    | 0.013 $\pm$ 0.001 | 0.24 $\pm$ 0.060  | 640.87 $\pm$ 22.2.323  | 1491.225 $\pm$ 2.77.28  | 66.918 $\pm$ 2.206      |
| 2/3                                  | 19.999 $\pm$ 2.498    | 653.076 $\pm$ 4.1.904 | 52.346 $\pm$ 2.152  | 1290.73 $\pm$ 8.4.133  | 44.500 $\pm$ 3.140  | 34.115 $\pm$ 3.902   | 723.307 $\pm$ 1.23.234 | 0.02 $\pm$ 0.02   | 0.377 $\pm$ 0.063 | 585.498 $\pm$ 5.2.645  | 1400.419 $\pm$ 2.61.195 | 71.967 $\pm$ 1.663      |
| 3                                    | 12.841 $\pm$ 4.275    | 485.432 $\pm$ 9.4.487 | 50.16 $\pm$ 5.554   | 1094.788 $\pm$ 290.255 | 40.525 $\pm$ 6.038  | 34.125 $\pm$ 1.0.168 | 727.528 $\pm$ 2.27.984 | 0.033 $\pm$ 0.014 | 0.326 $\pm$ 0.178 | 482.806 $\pm$ 1.22.641 | 1263.197 $\pm$ 3.17.058 | 73.371 $\pm$ 3.415      |
| 3/4                                  | 10.524 $\pm$ 1.836    | 317.961 $\pm$ 6.0.027 | 44.692 $\pm$ 6.442  | 552.500 $\pm$ 4.8.228  | 40.461 $\pm$ 3.679  | 43.692 $\pm$ 3.495   | 798.846 $\pm$ 2.01.968 | 0.041 $\pm$ 0.006 | 0.172 $\pm$ 0.072 | 485.001 $\pm$ 1.19.232 | 1211.163 $\pm$ 2.67.93  | 70.823 $\pm$ 3.722      |
| 4                                    | 10.729 $\pm$ 1.044    | 160.961 $\pm$ 1.2.327 | 56.576 $\pm$ 5.981  | 491.307 $\pm$ 3.1.820  | 51.653 $\pm$ 3.497  | 41.961 $\pm$ 3.304   | 303.692 $\pm$ 4.1.498  | 0.054 $\pm$ 0.009 | 0.55 $\pm$ 0.059  | 189.626 $\pm$ 4.9.54   | 501.304 $\pm$ 31.893    | 77.293 $\pm$ 2.247      |

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551

552 Table S4. Bonferroni post hoc tests accounting for species vs. species differences in each  
 553 sound parameter.

| Species i           | Species j              | Difference (i-j)    |                |                |                |                |                |          |                |          |                 |                 |                    |        |
|---------------------|------------------------|---------------------|----------------|----------------|----------------|----------------|----------------|----------|----------------|----------|-----------------|-----------------|--------------------|--------|
|                     |                        | PR                  | F <sub>1</sub> | I <sub>1</sub> | F <sub>2</sub> | I <sub>2</sub> | I <sub>3</sub> | CG       | Δ <sub>1</sub> | RMS      | Q <sub>25</sub> | Q <sub>75</sub> | %E <sub>Peak</sub> |        |
| <i>M. alcon</i>     | <i>C. argiades</i>     | -18.42*             | -318.23*       | 11.33*         | -525.13*       | 10.96*         | 1.82           | -643.47* | 0.04*          | 0.3*     | -442.04*        | -975.24*        | 9.96*              |        |
|                     | <i>P. argus</i>        | 0.34                | -146.87*       | 12.3*          | -63.07         | 11.37*         | -1.1           | -487.5*  | 0.01*          | 0.38*    | -307.97*        | -725.83*        | 6.88*              |        |
|                     | <i>L. argyrognomon</i> | -5.39*              | -374.53*       | 4.45*          | -589.97*       | 6.18*          | 2.57           | -509.9*  | 0.02*          | 0.27*    | -372.37*        | -789.95*        | 4.98*              |        |
|                     | <i>P. bellargus</i>    | -5.98*              | -362.27*       | 10.03*         | -646.67*       | 11.37*         | 7.8*           | -508.63* | 0.04*          | 0.35*    | -319.44*        | -840.3*         | 7.12*              |        |
|                     | <i>P. coridon</i>      | 1.37                | -219.37*       | 5.23*          | -350.93*       | 14.83*         | 11.97*         | -251.47* | 0              | 0.26*    | -177.83*        | -494.75*        | 0.43               |        |
|                     | <i>L. dispar</i>       | -13.55*             | -526.6*        | 9.21*          | -841.37*       | 8.37*          | 6.47*          | -746.67* | 0.04*          | 0.24*    | -527.77*        | -               | 9.51*              |        |
|                     | <i>L. helle</i>        | -5.66*              | -567.13*       | 2.33           | -913.2*        | 9.2*           | 1.73           | -640.57* | 0.03*          | 0.06     | -430.97*        | -               | 6.51*              |        |
|                     | <i>P. icarus</i>       | -9.19*              | -486.2*        | 3.5            | -788.83*       | 8.23*          | 9.2*           | -423.53* | 0.03*          | 0.17*    | -398.05*        | -917.82*        | 5.33*              |        |
|                     | <i>C. marshalli</i>    | -3.41*              | -355.97*       | 17.57*         | -565.4*        | 30.4*          | 36.62*         | -195.77* | 0.03*          | 0.47*    | -151.85*        | -183.97*        | 8.86*              |        |
|                     | <i>S. orion</i>        | 1.33                | -353.97*       | 1.8            | -919.03*       | 13.8*          | 12.87*         | -334.63* | 0.02*          | -0.01    | -276.32*        | -756.67*        | 2.93*              |        |
| <i>L. phleas</i>    | -15.55*                | -437.53*            | 15.12*         | -695.5*        | 11.15*         | 6.33*          | -790.33*       | 0.04*    | 0.37*          | -562.88* | -               | 13.45*          |                    |        |
|                     |                        |                     |                |                |                |                |                |          |                |          | 1286.96*        |                 |                    |        |
|                     |                        |                     |                |                |                |                |                |          |                |          | 1416.98*        |                 |                    |        |
|                     |                        |                     |                |                |                |                |                |          |                |          | 1099.08*        |                 |                    |        |
| <i>C. argiades</i>  | <i>P. argus</i>        | 18.76*              | 171.37*        | 0.97           | 462.07*        | 0.4            | -2.92          | 155.97*  | -0.03*         | 0.08*    | 134.08*         | 249.41*         | -3.07*             |        |
|                     | <i>L. argyrognomon</i> | 13.03*              | -56.3*         | -6.89*         | -64.83         | -4.78*         | 0.75           | 133.57*  | -0.02*         | -0.03    | 69.67           | 185.29*         | -4.98*             |        |
|                     | <i>P. bellargus</i>    | 12.44*              | -44.03         | -1.3           | -121.53*       | 0.4            | 5.98*          | 134.83*  | 0              | 0.05     | 122.61*         | 134.95          | -2.83*             |        |
|                     | <i>P. coridon</i>      | 19.79*              | 98.87*         | -6.1*          | 174.2*         | 3.87           | 10.15*         | 392*     | -0.04*         | -0.04    | 264.21*         | 480.5*          | -9.52*             |        |
|                     | <i>L. dispar</i>       | 4.87*               | -208.37*       | -2.12          | -316.23*       | -2.6           | 4.65           | -103.2   | 0              | -0.06    | -85.72          | -311.71*        | -0.45              |        |
|                     | <i>L. helle</i>        | 12.76*              | -248.9*        | -9*            | -388.07*       | -1.76          | -0.09          | 2.9      | -0.01*         | -0.24*   | 11.07           | -441.74*        | -3.45*             |        |
|                     | <i>P. icarus</i>       | 9.23*               | -167.97*       | -7.83*         | -263.7*        | -2.73          | 7.38*          | 219.93*  | -0.01*         | -0.13*   | 44              | 57.42           | -4.63*             |        |
|                     | <i>C. marshalli</i>    | 15.01*              | -37.73         | 6.23*          | -40.27         | 19.44*         | 34.8*          | 447.7*   | -0.01*         | 0.17*    | 290.19*         | 791.27*         | -1.1               |        |
|                     | <i>S. orion</i>        | 19.75*              | -35.73         | -9.53*         | -393.9*        | 2.84           | 11.05*         | 308.83*  | -0.02*         | -0.31*   | 165.72*         | 218.57*         | -7.03*             |        |
|                     | <i>L. phleas</i>       | 2.87*               | -119.3*        | 3.78           | -170.37*       | 0.19           | 4.51           | -146.87* | 0              | 0.07     | -120.84*        | -123.84         | 3.49*              |        |
| <i>P. argus</i>     | <i>L. argyrognomon</i> | -5.73*              | -227.67*       | -7.85*         | -526.9*        | -5.18*         | 3.67           | -22.4    | 0.01*          | -0.1*    | -64.41          | -64.12          | -1.91              |        |
|                     | <i>P. bellargus</i>    | -6.32*              | -215.4*        | -2.27          | -583.6*        | 0              | 8.9*           | -21.13   | 0.02*          | -0.02    | -11.47          | -114.46         | 0.24               |        |
|                     | <i>P. coridon</i>      | 1.03                | -72.5*         | -7.07*         | -287.87*       | 3.47           | 13.07*         | 236.03*  | -0.01*         | -0.12*   | 130.14*         | 231.09*         | -6.45*             |        |
|                     | <i>L. dispar</i>       | -13.89*             | -379.73*       | -3.09          | -778.3*        | -3             | 7.57*          | -259.17* | 0.03*          | -0.13*   | -219.8*         | -561.12*        | 2.62*              |        |
|                     | <i>L. helle</i>        | -6*                 | -420.27*       | -9.97*         | -850.13*       | -2.17          | 2.83           | -153.07* | 0.02*          | -0.31*   | -123.01*        | -691.15*        | -0.37              |        |
|                     | <i>P. icarus</i>       | -9.53*              | -339.33*       | -8.8*          | -725.77*       | -3.13          | 10.3*          | 63.97    | 0.02*          | -0.21*   | -90.08          | -191.98*        | -1.55              |        |
|                     | <i>C. marshalli</i>    | -3.75*              | -209.1*        | 5.27*          | -502.33*       | 19.03*         | 37.72*         | 291.73*  | 0.02*          | 0.09*    | 156.11*         | 541.86*         | 1.97               |        |
|                     | <i>S. orion</i>        | 0.99                | -207.1*        | -10.5*         | -855.97*       | 2.43           | 13.97*         | 152.87*  | 0.01*          | -0.38*   | 31.64           | -30.84          | -3.96*             |        |
|                     | <i>L. phleas</i>       | -15.89*             | -290.67*       | 2.82           | -632.43*       | -0.21          | 7.43*          | -302.83* | 0.03*          | -0.01    | -254.91*        | -373.25*        | 6.56*              |        |
|                     | <i>L. argyrognomon</i> | <i>P. bellargus</i> | -0.59          | 12.27          | 5.59*          | -56.7          | 5.18*          | 5.23     | 1.27           | 0.01*    | 0.08*           | 52.94           | -50.35             | 2.14   |
| <i>P. coridon</i>   |                        | 6.76*               | 155.17*        | 0.79           | 239.03*        | 8.65*          | 9.4*           | 258.43*  | -0.02*         | -0.02    | 194.55*         | 295.2*          | -4.54*             |        |
| <i>L. dispar</i>    |                        | -8.16*              | -152.07*       | 4.77*          | -251.4*        | 2.18           | 3.9            | -236.77* | 0.02*          | -0.03    | -155.39*        | -497.01*        | 4.53*              |        |
| <i>L. helle</i>     |                        | -0.27               | -192.6*        | -2.11          | -323.23*       | 3.02           | -0.84          | -130.67* | 0.01*          | -0.21*   | -58.6           | -627.03*        | 1.53               |        |
| <i>P. icarus</i>    |                        | -3.8*               | -111.67*       | -0.95          | -198.87*       | 2.05           | 6.63*          | 86.37    | 0.01*          | -0.11*   | -25.67          | -127.87         | 0.35               |        |
| <i>C. marshalli</i> |                        | 1.97                | 18.57          | 13.12*         | 24.57          | 24.22*         | 34.05*         | 314.13*  | 0.01*          | 0.19*    | 220.52*         | 605.98*         | 3.88*              |        |
| <i>S. orion</i>     |                        | 6.72*               | 20.57          | -2.65          | -329.07*       | 7.62*          | 10.3*          | 175.27*  | 0              | -0.28*   | 96.05*          | 33.28           | -2.05              |        |
| <i>L. phleas</i>    |                        | -10.16*             | -63*           | 10.67*         | -105.53*       | 4.97*          | 3.76           | -280.43* | 0.02*          | 0.09*    | -190.5*         | -309.13*        | 8.47*              |        |
| <i>P. bellargus</i> |                        | <i>P. coridon</i>   | 7.35*          | 142.9*         | -4.8*          | 295.73*        | 3.47           | 4.17     | 257.17*        | -0.04*   | -0.1*           | 141.61*         | 345.55*            | -6.69* |
|                     |                        | <i>L. dispar</i>    | -7.57*         | -164.33*       | -0.82          | -194.7*        | -3             | -1.33    | -238.03*       | 0        | -0.11*          | -208.33*        | -446.66*           | 2.38*  |
|                     | <i>L. helle</i>        | 0.32                | -204.87*       | -7.7*          | -266.53*       | -2.17          | -6.07*         | -131.93* | 0              | -0.29*   | -111.54*        | -576.69*        | -0.61              |        |
|                     | <i>P. icarus</i>       | -3.21*              | -123.93*       | -6.53*         | -142.17*       | -3.13          | 1.4            | 85.1     | 0              | -0.19*   | -78.61          | -77.52          | -1.79              |        |
|                     | <i>C. marshalli</i>    | 2.57*               | 6.3            | 7.53*          | 81.27          | 19.03*         | 28.82*         | 312.87*  | -0.01*         | 0.11*    | 167.58*         | 656.32*         | 1.73               |        |
|                     | <i>S. orion</i>        | 7.31*               | 8.3            | -8.23*         | -272.37*       | 2.43           | 5.07           | 174*     | -0.01*         | -0.36*   | 43.11           | 83.62           | -4.19*             |        |
|                     | <i>L. phleas</i>       | -9.57*              | -75.27*        | 5.08*          | -48.83         | -0.21          | -1.47          | -281.7*  | 0              | 0.01     | -243.44*        | -258.79*        | 6.33*              |        |
|                     | <i>P. coridon</i>      | <i>L. dispar</i>    | -14.92*        | -307.23*       | 3.98*          | -490.43*       | -6.47*         | -5.5     | -495.2*        | 0.04*    | -0.01           | -349.94*        | -792.21*           | 9.07*  |
| <i>L. helle</i>     |                        | -7.03*              | -347.77*       | -2.9           | -562.27*       | -5.63*         | -10.23*        | -389.1*  | 0.03*          | -0.19*   | -253.14*        | -922.23*        | 6.08*              |        |
| <i>P. icarus</i>    |                        | -10.56*             | -266.83*       | -1.73          | -437.9*        | -6.6*          | -2.77          | -172.07* | 0.03*          | -0.09*   | -220.22*        | -423.07*        | 4.9*               |        |
| <i>C. marshalli</i> |                        | -4.78*              | -136.6*        | 12.33*         | -214.47*       | 15.57*         | 24.65*         | 55.7     | 0.03*          | 0.21*    | 25.98           | 310.77*         | 8.42*              |        |
| <i>S. orion</i>     |                        | -0.04               | -134.6*        | -3.43          | -568.1*        | -1.03          | 0.9            | -83.17   | 0.02*          | -0.26*   | -98.49*         | -261.93*        | 2.5*               |        |
| <i>L. phleas</i>    |                        | -16.92*             | -218.17*       | 9.88*          | -344.57*       | -3.68          | -5.64          | -538.87* | 0.04*          | 0.11*    | -385.05*        | -604.33*        | 13.01*             |        |
| <i>L. dispar</i>    |                        | <i>L. helle</i>     | 7.89*          | -40.53         | -6.88*         | -71.83         | 0.83           | -4.73    | 106.1          | 0        | -0.18*          | 96.79*          | -130.03            | -3*    |
|                     | <i>P. icarus</i>       | 4.36*               | 40.4           | -5.71*         | 52.53          | -0.13          | 2.73           | 323.13*  | -0.01*         | -0.07*   | 129.72*         | 369.14*         | -4.18*             |        |
|                     | <i>C. marshalli</i>    | 10.14*              | 170.63*        | 8.35*          | 275.97*        | 22.03*         | 30.15*         | 550.9*   | -0.01*         | 0.23*    | 375.91*         | 1102.98*        | -0.65              |        |
|                     | <i>S. orion</i>        | 14.88*              | 172.63*        | -7.41*         | -77.67         | 5.43*          | 6.4*           | 412.03*  | -0.02*         | -0.25*   | 251.45*         | 530.28*         | -6.58*             |        |
|                     | <i>L. phleas</i>       | -2                  | 89.07*         | 5.9*           | 145.87*        | 2.79           | -0.14          | -43.67   | 0              | 0.12*    | -35.11          | 187.87*         | 3.94*              |        |
| <i>L. helle</i>     | <i>P. icarus</i>       | -3.53*              | 80.93*         | 1.17           | 124.37*        | -0.97          | 7.47*          | 217.03*  | 0              | 0.1*     | 32.93           | 499.16*         | -1.18              |        |
|                     | <i>C. marshalli</i>    | 2.25                | 211.17*        | 15.23*         | 347.8*         | 21.2*          | 34.89*         | 444.8*   | 0              | 0.4*     | 279.12*         | 1233.01*        | 2.35*              |        |
|                     | <i>S. orion</i>        | 6.99*               | 213.17*        | -0.53          | -5.83          | 4.6*           | 11.13*         | 305.93*  | -0.01*         | -0.07    | 154.65*         | 660.31*         | -3.58*             |        |
|                     | <i>L. phleas</i>       | -9.89*              | 129.6*         | 12.78*         | 217.7*         | 1.95           | 4.59           | -149.77* | 0              | 0.3*     | -131.91*        | 317.9*          | 6.94*              |        |

|                     |                     |         |         |         |          |         |         |          |        |        |          |          |        |
|---------------------|---------------------|---------|---------|---------|----------|---------|---------|----------|--------|--------|----------|----------|--------|
| <i>P. icarus</i>    | <i>C. marshalli</i> | 5.77*   | 130.23* | 14.07*  | 223.43*  | 22.17*  | 27.42*  | 227.77*  | 0      | 0.3*   | 246.19*  | 733.84*  | 3.53*  |
|                     | <i>S. orion</i>     | 10.52*  | 132.23* | -1.7    | -130.2*  | 5.57*   | 3.67    | 88.9     | -0.01* | -0.17* | 121.72*  | 161.15   | -2.4*  |
|                     | <i>L. phleas</i>    | -6.36*  | 48.67*  | 11.62*  | 93.33    | 2.92    | -2.87   | -366.8*  | 0.01*  | 0.2*   | -164.83* | -181.26  | 8.12*  |
| <i>C. marshalli</i> | <i>S. orion</i>     | 4.74*   | 2       | -15.77* | -353.63* | -16.6*  | -23.75* | -138.87* | -0.01* | -0.47* | -124.47* | -572.7*  | -5.93* |
|                     | <i>L. phleas</i>    | -12.14* | -81.57* | -2.45   | -130.1*  | -19.25* | -30.29* | -594.57* | 0.01*  | -0.1*  | -411.03* | -915.11* | 4.59*  |
| <i>S. orion</i>     | <i>L. phleas</i>    | -16.88* | -83.57* | 13.32*  | 223.53*  | -2.65   | -6.54*  | -455.7*  | 0.02*  | 0.37*  | -286.56* | -342.41* | 10.52* |

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556 Table S4. Bonferroni post hoc tests accounting for differences in each sound parameter  
 557 between myrmecophilous categories (0= myrmecoxenous; 2= moderately myrmecophilous; 3=  
 558 steadily myrmecophilous; 4= obliged myrmecophilous)

|          |          | Difference (i-j) |                |                |                |                |                |         |                |        |                 |                 |                     |
|----------|----------|------------------|----------------|----------------|----------------|----------------|----------------|---------|----------------|--------|-----------------|-----------------|---------------------|
| Cat<br>i | Cat<br>j | PR               | F <sub>1</sub> | I <sub>1</sub> | F <sub>2</sub> | I <sub>2</sub> | I <sub>3</sub> | CG      | Δ <sub>t</sub> | RMS    | Q <sub>25</sub> | Q <sub>75</sub> | %E <sub>Fpeak</sub> |
| 0        | 2        | -8.88*           | 153.58*        | 0.28           | 228.73*        | -3.82          | -10.97*        | -50.13  | 0              | 0.02   | -23.67          | 21.51           | 0.38                |
|          | 2/3      | 0.36             | -14.39         | -7.56*         | -34.97         | -6.55*         | -3.59          | 169.8*  | 0              | -0.12* | 20.32           | 78.93           | -4.25*              |
|          | 3        | 7.38*            | 144.28*        | -5.68*         | 127.22*        | -3.23*         | -3.99          | 192.18* | -0.02*         | -0.07* | 131.88*         | 276.33*         | -5.71*              |
|          | 3/4      | 9.88*            | 324.94*        | 1.24           | 690.8*         | -3.41          | -13.89*        | 105.83  | -0.02*         | 0.09   | 110.4*          | 270.91*         | -2.7*               |
|          | 4        | 9.54*            | 471.81*        | -11.06*        | 753.87*        | -14.78*        | -12.79*        | 593.33* | -0.04*         | -0.28* | 418.37*         | 996.75*         | -9.58*              |
| 2        | 2/3      | 9.23*            | -167.97*       | -7.83*         | -263.7*        | -2.73          | 7.38           | 219.93* | -0.01          | -0.13* | 44              | 57.42           | -4.63*              |
|          | 3        | 16.25*           | -9.3           | -5.96*         | -101.52        | 0.58           | 6.98*          | 242.31* | -0.02*         | -0.08  | 155.55*         | 254.83*         | -6.09*              |
|          | 3/4      | 18.76*           | 171.37*        | 0.97           | 462.07*        | 0.4            | -2.92          | 155.97  | -0.03*         | 0.08   | 134.08*         | 249.41          | -3.07*              |
|          | 4        | 18.42*           | 318.23*        | -11.33*        | 525.13*        | -10.96*        | -1.82          | 643.47* | -0.04*         | -0.3*  | 442.04*         | 975.24*         | -9.96*              |
| 2/3      | 3        | 7.02*            | 158.67*        | 1.88           | 162.18*        | 3.31           | -0.4           | 22.38   | -0.01*         | 0.05   | 111.56*         | 197.4           | -1.46               |
|          | 3/4      | 9.53*            | 339.33*        | 8.8*           | 725.77*        | 3.13           | -10.3*         | -63.97  | -0.02*         | 0.21*  | 90.08           | 191.98          | 1.55                |
|          | 4        | 9.19*            | 486.2*         | -3.5           | 788.83*        | -8.23*         | -9.2*          | 423.53* | -0.03*         | -0.17* | 398.05*         | 917.82*         | -5.33*              |
| 3        | 3/4      | 2.51             | 180.67*        | 6.92*          | 563.58*        | -0.18          | -9.9*          | -86.34  | -0.01*         | 0.16*  | -21.48          | -5.42           | 3.02*               |
|          | 4        | 2.17             | 327.53*        | -5.38*         | 626.65*        | -11.55*        | -8.8*          | 401.16* | -0.02*         | -0.22* | 286.49*         | 720.42*         | -3.87*              |
| 3/4      | 4        | -0.34            | 146.87*        | -12.3*         | 63.07          | -11.37*        | 1.1            | 487.5*  | -0.01*         | -0.38* | 307.97*         | 725.83*         | -6.88*              |

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