

1 Title: Effect of host species, host nest density and nest size on the occurrence of the shining guest ant
2 *Formicoxenus nitidulus* (Hymenoptera: Formicidae)

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10

11 Abstract: Understanding habitat requirements of species is important in conservation. As an obligate ant nest
12 associate, the survival of the globally vulnerable shining guest ant, *Formicoxenus nitidulus*, is strictly tied to that
13 of its hosts (mound building *Formica* ants). We investigated how host species, nest density, inter-nest distance
14 and nest mound size relate to the occurrence of *F. nitidulus*. In total, 166 red wood ant nests were surveyed in
15 SW Finland (120 *Formica polyctena*, 25 *F. rufa*, 14 *F. aquilonia*, 5 *F. pratensis*, and 2 *F. lugubris*). Overall, *F.*
16 *nitidulus* was found in 60 % of the nests. For the actual analysis, only *F. polyctena* and *F. rufa* nests were
17 included due to the small number of other nests. *F. nitidulus* was more likely to be found among *F. polyctena*
18 than *F. rufa*. Also, while inter-nest distance was not important, a high nest density, commonly found in
19 polydomous (multi-nest) wood ant colonies, was beneficial for *F. nitidulus*. The guest ant was also more likely
20 to be found in large host nests than small nests. Thus, our results show that the best habitat for the guest ant is a
21 dense population of host nest mounds with a high proportion of large mounds. Conservation efforts should be
22 directed at keeping the quality of the red wood ant habitats high to preserve their current populations and to
23 increase colonization. This will not only benefit the guest ant, but also a plethora of other species, and help in
24 maintaining the biodiversity of forests.

25

26 Keywords: *Formicoxenus nitidulus*, *Formica*, social parasite, xenobiosis, conservation, metapopulation

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28

29 Introduction

30

31 Human induced habitat loss and fragmentation are a serious threat to the persistence of
32 numerous species (e.g. Tschardt et al. 2002; Fahrig 2003; Van Swaay et al. 2005; Van
33 Dyck et al. 2009). Moreover, inappropriate management of existing habitats can be the cause
34 of further population declines (Balmer and Erhardt 2000; Waring 2001; Mabelis and
35 Korczyńska 2016). To effectively direct conservation efforts, it is essential to understand the
36 habitat requirements of species (Thomas et al. 2009). Special care must be taken when the
37 species under consideration are narrowly specialized.

38

39 Parasites are expected to decline or go extinct when the host population size decreases below
40 a critical threshold density (Altizer et al. 2007). They can also be more affected by area loss
41 and increased isolation than their hosts, as shown in host-parasitoid systems (e.g. van
42 Nouhuys and Hanski 1999). Furthermore, in the special case of social parasitism (here
43 referred to as parasite-host relationship between two social insect species), parasitic cuckoo
44 bumblebees were more vulnerable to extinction than their host bumblebee species (Suhonen
45 et al. 2015). Most social parasites are rare and often occupy only small parts of the range of
46 the host species (e.g. Hölldobler and Wilson 1990; Zamora-Muñoz et al. 2003; Buschinger
47 2009).

48

49 Social parasitism is especially manifold among ants where four basic types of obligatory
50 parasitic associations can be recognised: xenobiosis, temporary parasitism (occurs during
51 colony foundation), permanent parasitism with slavery (dulosis) and without slavery
52 (inquilinism) (Buschinger 2009). Ants of the genus *Formicoxenus* (Hymenoptera, Formicidae,
53 Myrmicinae) are xenobionts, so called guest ants, which live freely within the host colony

54 and, unlike other social parasites, care for their own brood (Buschinger 2009). Social parasites
55 often rely on chemical mimicry to blend into the host colony, and are thus, also highly host
56 specific (Errard & al. 1997, Lenoir & al. 1997). However, the shining guest ant,
57 *Formicoxenus nitidulus* (Nylander 1846), employs an entirely different strategy, which allows
58 it to invade the nests of at least nine different host ant species of the genus *Formica* (*F. rufa*
59 Linnaeus 1761, *F. polychtena* Förster 1850, *F. aquilonia* Yarrow 1955, *F. pratensis* Retzius
60 1783, *F. lugubris* Zetterstedt 1838, *F. truncorum* Fabricius 1804, *F. uralensis* Ruzsky 1895,
61 *F. exsecta* Nylander 1846, and *F. pressilabris* Nylander 1846) (Francoeur et al. 1985; Busch
62 2001; Czechowski et al. 2002). Due to chemical deterrents in its cuticle (Martin et al. 2007),
63 the guest ant is mostly treated with indifference by its hosts. In the rare occasion the guest ant
64 is grabbed by a host worker, it is immediately dropped (Robinson 2005; Martin et al. 2007).

65

66 We studied the occurrence of *F. nitidulus* in Finland in the nests of five species of red wood
67 ants (*Formica rufa* group): *F. rufa*, *F. polychtena*, *F. aquilonia*, *F. lugubris* and *F. pratensis*;
68 which are dominant insects in the boreal forests of Eurasia (e.g. Rosengren and Pamilo 1983).
69 They build large long-lived mound nests consisting of forest litter and resin and are
70 considered as keystone species in the forest ecosystem with ecological effects extending over
71 several trophic levels (see Sorvari et al. 2011). Furthermore, they have an important role as
72 host species to a wide array of other invertebrates, i.e. myrmecophiles, in addition to the guest
73 ants (e.g. Härkönen and Sorvari 2014, Parmentier et al. 2014, Robinson et al. 2016).

74

75 The number of queens and nests in a red wood ant colony varies considerably within and
76 between species (Ellis and Robinson 2014). In Finland, most populations of *F. rufa*, *F.*
77 *lugubris* and *F. pratensis* are monogynous (one queen) and monodomous (single nest colony),
78 while *F. polychtena* and *F. aquilonia* are polygynous (several queens per colony) and

79 polydomous (multi-nest colonies) (Rosengren and Pamilo 1983). Red wood ants also differ in
80 their dispersal strategies. Consequently, *Formica polyctena* thrives in areas where there are
81 large continuous forests while *F. rufa* is better at dispersing to small and isolated woodland
82 patches, and thus, is better adapted to habitat fragmentation (Rosengren et al. 1993; Punttila
83 1996; Sundström et al 2005; Mabelis and Korczyńska 2016).

84

85 *Formicoxenus nitidulus* has a wide distribution ranging throughout most of Europe and into
86 Eastern Siberia (Collingwood 1979; Agosti and Collingwood 1987; Czechowski et al. 2002).
87 Although the guest ant is quite common in Finland (Rassi et al. 2010), globally it has been
88 classified as vulnerable according to the IUCN Red List (IUCN 2015). Naturally, the survival
89 of the guest ant is intimately tied to the survival of its hosts. However, not all of the potential
90 host nests are occupied by the guest ant. The nests of the red wood ants can be seen as
91 suitable habitat patches surrounded by uninhabitable landscape for various obligate
92 associates, including guest ants. Patch occupancy can be predicted by different parameters
93 such as patch size, patch isolation and habitat quality (e.g. Kindvall and Ahlén 1992; Hanski
94 1999; Thomas et al. 2001; Eichel and Fartmann 2008). Based on metapopulation theory, small
95 and isolated patches (or nest mounds) are expected to have a higher risk of extinction due to
96 smaller carrying capacities and fewer chances of colonization when empty (Hanski 1999).

97 Previous guest ant studies have mostly focused on *F. nitidulus* occurrence in regards to the
98 prevailing host nest conditions, and show the guest ants preferring larger and more evenly
99 built nest mounds with a higher mean temperature (Dietrich 1997; Ölzant 2001).

100

101 Also, the different colony structures of the host species likely play a role in the guest ant
102 occurrence. Young *F. nitidulus* queens disperse in late summer flying or walking, as in the
103 case of wingless intermorphs (intermediate forms between regular workers and winged

104 queens). *Formicoxenus nitidulus* is able to use the scent trails left by its host for orientation
105 (Elgert and Rosengren 1977), and thus, the trails connecting nests in a polydomous colony
106 provide easy pathways for them to follow while dispersing. This would increase the chance of
107 survival for the local guest ant population in a polydomous colony.

108

109 In this study, we investigated how host species, host nest density, inter-nest distance, and nest
110 size, relate to the occurrence of the guest ant *F. nitidulus*. As nests tend to be larger and nest
111 density higher among polydomous red wood ants (Punntila and Kilpeläinen 2009), we expect
112 their nests to be more likely occupied by the guest ant than the nests of monodomous hosts.
113 We also discuss our results in the context of conservation of this species, while considering
114 the differences in the host species.

115

116 Material and methods

117

118 Study species

119 The shining guest ant *F. nitidulus* is a tiny (~2.8 - 3.4 mm) Myrmecine ant easily identified
120 from their much larger hosts (Fig.1). They are most easily detected from late summer to
121 autumn (Robinson 1999; Van Hengel 2011) after mating has occurred on top of the nest
122 mound, and most often it is the males that are seen. Instead of dying shortly after mating, the
123 males continue to come to the surface of the nest for the rest of the season. Unlike in most
124 other ant species, *F. nitidulus* males are wingless and very worker-like in appearance though
125 they have 12 antennal segments while workers and queens have 11 (Fig. 2; key in
126 Collingwood 1979). Workers are seen more rarely as they tend to stay hidden within the nest
127 mound.

128

129 Field work

130 The field work was carried out in Turku, SW Finland (60°25'N, 22°09'E), in June-September
131 2014. We surveyed 166 red wood ant nests (120 *Formica polycтена*, 25 *F. rufa*, 14 *F.*
132 *aquilonia*, 5 *F. pratensis*, and 2 *F. lugubris*) in 25 sites on the presence of *Formicoxenus*
133 *nitidulus*. The sites were mostly in conifer and mixed forests, but there were a couple of sites
134 in herb-rich oak forests. Both sites with high nest density and sites with low nest density were
135 chosen for this study, as well as couple of sites with single isolated nests. Twelve sites were
136 occupied by *F. polycтена*, five by *F. rufa*, one by *F. pratensis*, and one by *F. aquilonia*. On
137 the remaining six sites, *F. polycтена* occurred together with *F. rufa*, *F. pratensis*, and/or *F.*
138 *lugubris*.

139

140 Nest mounds were systematically observed during August – September as this is the best time
141 for detecting the guest ants. Each nest was observed a maximum of 10 minutes before moving
142 on to the next one. This has been found to be a sufficient time to detect the guest ant when
143 they are present (Robinson 1998; Green and Westwood 2006). Despite their small size, the
144 shiny appearance and rapid movements of these ants make them relatively easy to spot against
145 the matte background of the nest mound.

146

147 Most of the nests in this study were also sampled (N = 135). Sampling of the nests started in
148 June, before the systematic observations. In a few cases the guest ants were seen on top of the
149 nests already in July while on a sampling round. July 3 marked the first such occasion when a
150 mating occurrence was observed on one of the *F. polycтена* nests. For sampling, nests were
151 divided into seven size classes based on the above ground nest volumes (< 250 l, 250 l – 750
152 l, 750 l – 1250 l, ..., 2250 l – 2750 l, 2750 l <). The guest ants are not likely to be equally
153 distributed within the nest mounds and with increasing nest size the amount of guest ant free

154 space is also likely to increase making it less likely for the guest ants to end up in a sample.
155 Thus, in an effort to counteract this, we increased the amount of nest material taken from the
156 nests based on their size class (1-7 x 0.5 l). Samples were taken about 5 cm beneath the outer
157 layer of the mound and then sieved with a 2.5 mm sieve. The coarse material left on top of the
158 sieve was looked through in the field and returned to the nest while the fine material was
159 brought into the laboratory for later examination.

160

161 Two variables were used to describe mound isolation: nest density and nearest neighbour
162 distance. To measure nest density, we counted the number of all potential host nests within a
163 100 metre radius of each observed nest. Inter-nest distances were calculated between all nest
164 locations from all sites based on their coordinates. The distance to the nearest neighbouring
165 nest (of a potential host species) was recorded for each nest. If there were no nests within 100
166 metres, the search was continued until a nest was found. Seven nests had a longer than 150 m
167 distance to the nearest neighbour. For these nests, the recorded distance might be inaccurate
168 as the scanning of the environment was more cursory after that distance, so there might have
169 been closer nests which were not found.

170

171 Above ground nest volumes were estimated by first measuring the height and diameter of
172 each nest mound and then using the equation for a half ellipsoid: $V = (4/3 \pi abc)/2$, where a, b
173 and c are the lengths of the semi-axes of the ellipsoid. The surrounding habitat of each nest
174 was described as either forest edge or forest interior (≤ 5 m and > 5 m from the edge
175 respectively). Weather on each day was classified as either mostly sunny or mostly cloudy.

176

177 Statistical analyses

178 Only *Formica polyctena* (N = 120) and *F. rufa* (N = 25) nests were included into the
179 statistical analyses, as there were so few of the other species nests. Also, as the sampling
180 method proved very inefficient, we focused only on observational data. All statistical analyses
181 were made with statistical software SAS version 9.3.

182

183 We used the generalized linear mixed model in the GLIMMIX procedure with binomial
184 distribution and logit link function to determine how the host species, nest density, distance to
185 the neighbouring nest, and mound size relate to the occurring probability of the guest ant. The
186 occurrence of *F. nitidulus* was used as a dependent variable (presence = 1, absence = 0) and
187 host species, nest density (measured as number of nests within 100 m radius from the focal
188 nest), mound size, distance to the neighbouring nest, and their interactions as fixed effects. In
189 addition, observation date and site were included as random factors with Kenward-Roger
190 approximation method for the degrees of freedom. Since inter-nest distance and nest density
191 were correlated (Pearson's $r = -0.45$, $P < 0.0001$, Spearman's $\rho = -0.69$, $P < 0.0001$), they
192 were placed in two separate models. All interactions were non-significant and were excluded
193 from the models. As the models were unable to estimate AIC values, the best model was
194 selected by comparing the Pearson's correlation coefficients (r) between the predicted values
195 of the competing models and the explanatory variable by which the models differed (distance
196 and nest density). Since the variables were not normally distributed, also the Spearman's non-
197 parametric correlations were compared.

198

199 We used the MIXED procedure to see whether the surrounding habitat of the nests was
200 connected to nest volume and nest density. Thus, two models were made, where surrounding
201 habitat of the nest (forest edge / interior) was used as an explanatory variable with either nest
202 volume or nest density as a dependent variable.

203

204 Since nest volume and nest density were strongly dependent on the surroundings of the nest
205 (forest edge / interior), and weather (sunny/cloudy) was connected to date, a separate model
206 (GLIMMIX: binomial distribution, logit link function) was used to test the effects of nest
207 surrounding habitat and weather on guest ant occurrence, with *F. nitidulus* occurrence as the
208 dependent variable and nest habitat and weather as fixed effects.

209

210 Results

211

212 Of the 166 red wood ant mounds we surveyed, ca. 60 % were inhabited by *F. nitidulus* (Table
213 1). Mostly when the guest ants were found to be present, they were seen within the first few
214 minutes of observation. We detected no guest ants in the studied nests of *F. pratensis* and *F.*
215 *lugubris*.

216

217 Maximum daytime temperature during observation days ranged from 14°C to 26°C (mean =
218 20°C, SD = 3.2), which is well within the temperature range at which the guest ants (males)
219 can be found on the nest mounds (Van Hengel 2011). The guest ants may be observed on the
220 nest mounds throughout the day; some reports indicating that mornings are best and others
221 vouching for afternoons (Ölzant 2001; Van Hengel 2011). In this study, observations were
222 mostly made in the afternoons starting around 13:00 and lasting until 15:00–16:00. There
223 were a couple of days when observations were started earlier at around 11. Exact times were
224 not recorded but based on the order in which nests were visited on each site, there was no
225 apparent pattern in detection success within a day of observation.

226

227 Overall, the sampling method used was very inefficient in detecting the guest ants, as it gave a
228 positive result in only 23 % of the sampled nests. Also, sampling was successful in only ~39
229 % of the cases where guest ants were found by observation. There were also no nests where
230 the guest ants were found only by sampling. Failure to get the guest ants in a sample could be
231 the result of not sampling deep enough, as they might be more concentrated deeper in the
232 nest. Alternatively, trying to focus the sampling at nest openings might also be helpful, as
233 Busch (2001) found workers lurking just within when looking with a flashlight. However, it's
234 not always easy to see clear nest openings, especially in nests with a coarser surface structure.

235

236 All the extremely large nests belonged to either *F. polycytena* or *F. aquilonia*. The nest density
237 within 100 m radius of the focal nest ranged from zero to 24 nests, obviously being highest
238 with the polydomous species (Table 1). Correspondingly, inter-nest distances were generally
239 lower with the polydomous species (Table 1). There was also a lot of variation in nest
240 volumes, ranging from 4.7 to 2915.4 litres (Table 1).

241

242 Though the proportion of nests occupied by *F. nitidulus* was generally higher high nest
243 density and large nests (Fig. 3), the guest ants were also found in isolated and very small nests
244 (smallest ~20 l). According to both Pearson's and Spearman's correlation coefficients, the
245 better of the two competing models was the one with nest density instead of distance (Table
246 2). *F. nitidulus* was more likely to occur with *F. polycytena* than with *F. rufa* (Table 2, Fig. 4).
247 There was a significant positive relationship between host nest density and the presence of the
248 guest ant (Table 2, Fig. 4a). Also, nest volume was significantly and positively correlated with
249 the occurrence of the guest ant (Table 2, Fig. 4b).

250

251 Nest mounds were smaller ($F_{1, 143} = 5.02$, $P = 0.027$) and nest density lower ($F_{1, 143} = 20.81$, P
252 < 0.0001) along forest edges than inside the forests. The guest ants were more likely to be
253 found in nests that were inside the forest than on the edges ($F_{1, 142} = 8.07$, $P = 0.005$). Majority
254 of the nests (108) were observed in mostly sunny weather (with scattered clouds) and the
255 guest ants were found on 63 % of these. There were 37 nests which were observed in partly
256 cloudy to cloudy weather, of which 78 % were found to be occupied by the guest ant.
257 Nevertheless, there was no significant difference in guest ant occurrence between sunny and
258 cloudy days ($F_{1, 142} = 1.33$, $P = 0.251$).

259

260 Discussion

261

262 We found the guest ant *F. nitidulus* to be quite common in the study area in SW Finland. The
263 red wood ant *F. polyctena* was by far the most common of the host species in the area. With
264 75 % of its nests being occupied by the guest ants, it was also the most likely host. In contrast,
265 the guest ants were found in barely a third of the *F. rufa* nests. The guest ants were also more
266 likely to be found in well-connected nests (i.e. nests surrounded by high nest density) as well
267 as large nests. As these characteristics tend to be more usual among polydomous and
268 polygynous species (such as *F. polyctena*) than monodomous and monogynous species (such
269 as *F. rufa*) (e.g. Czechowski et al. 2002; Punttila and Kilpeläinen 2009), colony structure of
270 the host species plays an important role in the guest ant occurring probability. Particularly, the
271 trails increasing connectivity between the nests in a polydomous colony seem to contribute to
272 the high rate of occupancy among *F. polyctena*. Generally in ant social parasites, Buschinger
273 (2009) estimates the rate of parasitism to be much lower, somewhere between three and ten
274 percent of colonies parasitized within patches where the parasite is present.

275

276 Our results are consistent with the predictions of metapopulation theory (Hanski 1999). The
277 guest ant was more likely to be found in nests surrounded by a high nest density, as opposed
278 to more isolated nests. A similar result, where isolation explains patch occupancy, has been
279 observed in several other studies in insects (e.g. Kindvall and Ahlén 1992; Thomas and
280 Harrison 1992; Appelt and Poethke 1997; Thomas et al. 2001; Carlsson and Kindvall 2001;
281 Eichel and Fartmann 2008). Though published data are scarce, socially parasitic ants usually
282 occur in more or less isolated patches within the host range, and the patches are characterized
283 by a high density of the host species (Buschiger 2009). Also, previous studies on
284 myrmecophiles in red wood ant nests have reported a negative correlation between
285 myrmecophile diversity and host mound isolation (Päivinen et al. 2004; Härkönen and Sorvari
286 2014; Parmentier et al. 2015). Patch networks have to be sufficiently linked by dispersing
287 individuals to ensure the survival of species within them (Fahrig and Merriam 1985; Adler
288 and Nuernberger 1994; Hanski 1999; Bowne and Bowers 2004). When local populations
289 become extinct, recolonization relies on the amount of dispersing individuals and the ease of
290 movement within the landscape (Kindlmann and Burel 2008). A high nest density will
291 facilitate the dispersal to a new host nest.

292

293 Moreover, in a polydomous red wood ant colony dispersal can further be aided by trails
294 connecting the nests. Nests of monodomous red wood ants, on the other hand, might be harder
295 to find, even when inter-nest distances are relatively short, as they are not similarly connected
296 to other nests. Our results support this theory. On a *F. rufa* site where five nests were within
297 77 m of each other, and each had ≤ 27 m to a nearest neighbouring nest, only one nest was
298 found occupied by *F. nitidulus*. On another site, where *F. rufa* nests were relatively close to
299 several *F. polycytena* nests inhabited by *F. nitidulus*, the *F. rufa* nests were all without the
300 guest ant. The same was true for *F. pratensis* and *F. lugubris* nests even when they were fairly

301 close to guest ant inhabited *F. polyctena* nests. Similarly, Van Hengel (2011) reported that *F.*
302 *nitidulus* could be found in nearly all the nests in one *F. polyctena* super-colony while the
303 species was absent from all but one of the nearby *F. rufa* and *F. pratensis* nests. These
304 observations suggest that *F. nitidulus* prefers dispersing along the connecting trails, which
305 might be especially true for the wingless intermorphic females. Winged females, on the other
306 hand, are likely in a key position when dispersing to more isolated nests. For intermorphic
307 females, leaving a nest that is not directly connected to another nest might be much riskier. In
308 such cases, it seems it would be more prudent for the flightless queens to remain in the same
309 nest, though whether this is the case requires further study.

310

311 We confirm the previous finding of *F. nitidulus* being more likely to occur in larger nests than
312 smaller ones (Dietrich 1997; Ölzant 2001). Populations in large nests (patches) are less likely
313 to get extinct due to larger carrying capacities (Hanski 1999). Similarly, also the diversity of
314 myrmecophilous beetles has been found to be higher in large red wood ant nests (Päivinen et
315 al. 2004). Not only do the large nests provide the guest ants, as well as other guest species,
316 with more resources (Päivinen et al. 2004), larger nest mounds are also better able to buffer
317 against weather fluctuations and keep the inner temperature optimal, and thus have a more
318 stable microclimate (Hölldobler and Wilson 1990). Since ants are ectotherms, their growth
319 and reproduction is affected by the temperature of their habitat (Ratte 1984; Atkinson 1994;
320 Chown and Nicolson 2004). Large nests are also usually older and have been around longer
321 for the guest species to find and end up in by chance.

322

323 As the nests were observed only once during this study, it is possible that the guest ant was
324 not detected in all the nests where it is present. Probability to detect the guest ants is likely to

325 be affected by their population size in the host nest mound. Thus, the possible false-negative
326 observations could come from nest mounds with only few guest ant inhabitants.

327

328 Conservation perspectives

329 The Red List status of the guest species is based on the assessment of the IUCN Social Insects
330 Specialist Group from 1996 and requires revision. Recently, intensified ant inventories have
331 resulted in several new records of the species in Belgium and France after decades of no
332 observations (Wegnez et al. 2011). Also in UK, intensified searches have resulted in new
333 records of the species (UK Biodiversity Group 1999; Green 2009). Compared to many other
334 ant species, *F. nitidulus* is much harder to find and may thus be underrepresented in surveys
335 unless special attention is paid to the habits of the species. Due to its elusive lifestyle, to
336 maximise the chances of finding this guest ant, surveys should be made from late summer to
337 autumn with the best time usually being from August to September when the males are most
338 likely to be seen on top of the nest mounds (Ölzant 2001; Van Hengel 2011; Wegnez et al.
339 2011).

340

341 According to the IUCN Red List (IUCN, 2015), most of the host species of *F. nitidulus* are
342 Near Threatened (NT). This is due to the loss of suitable scrub and forest habitats for the host
343 species caused by agricultural clearing and inappropriate forest management practices. Wood
344 ants seem to be vulnerable even when modern forest management practices are used (Sorvari
345 and Hakkarainen 2007). Though clear-felling may temporarily increase nest mound density
346 due to the frequent establishment of new bud nests (Rosengren and Pamilo 1978; Rosengren
347 et al. 1979; Sorvari and Hakkarainen 2005), most nests, both old and new, will be abandoned
348 by the wood ants within a few years of the clear-cutting (Sorvari and Hakkarainen 2007). One
349 crucial factor causing nest abandonment is the distance of the nest mound to the remaining

350 forest, i.e., nests that are relatively close to the forest edge have a better chance to survive
351 (Sorvari 2013). Therefore, small size clearings may not be deleterious for red wood ant
352 colonies, and their associates.

353

354 *Formicoxenus nitidulus* is strictly dependent on its host species for survival, and like the
355 socially parasitic cuckoo bumblebees (Suhonen et al 2015), it is likely more vulnerable to
356 extinction than its hosts. For *F. nitidulus*, as well as for other obligate ant nest associates, the
357 best way to protect them is to ensure the survival of their hosts. Ants of the *Formica rufa*
358 group, which are the main hosts of the shining guest ant, are protected by law in many
359 European countries (IUCN 2015; Sorvari 2016). Per our results, the best habitat for the
360 shining guest ant is a dense population of mounds, with a high proportion of large mounds.
361 These parameters are more easily satisfied among polydomous host colonies, where trails
362 further increase connectivity. However, while large nest mounds are most optimal for the
363 shining guest ant, the small and medium sized nest mounds ensure the continuum of large
364 nests in a population also in the future.

365

366 Maintaining healthy populations of polydomous red wood ants requires the management of
367 sufficiently large (at least ≥ 25 ha) forest areas (Mabelis and Korczyńska 2016). However, in
368 many fragmented areas, most woodland patches are smaller, and thus better suited to
369 monogynous species (Punntila, 1996). Habitat quality is one of the crucial factors affecting
370 species persistence (e.g. Dennis and Eales 1997; Thomas et al. 2001). In Central Europe, the
371 quality of small woodland patches bordering agricultural land may be deteriorated increasing
372 the chances of extinction for wood ant populations (Mabelis and Korczyńska 2016). Thus, it is
373 essential to keep the quality of the woodland patches high to preserve the wood ants and their
374 various guest species. Red wood ants prefer to build their nests in sunny and open areas

375 within forests and along the edges (Mabelis and Korckzyńska 2016). To increase the chances
376 of colonization for red wood ants in managed forests, which are often dense, small open areas
377 could be created. This will create habitats for many other forest species as well. Thus,
378 maintaining a varied forest structure could help maintain or even increase the biodiversity of
379 forests.

380

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387

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557

558 **Table 1** Number of observed *Formica* host ant nests and the mean, SD, minimum, maximum,
559 and median values of parameters for *Formica* nests: nest mound volume (litres), nest density
560 (number of nests / 100 m radius of the focal nest), and distance to the nearest neighbouring
561 nest (metres); the number and percentage of observed nests in which the guest ant
562 *Formicoxenus nitidulus* was found are indicated within parentheses.

Host ant	Observed nests (<i>F. nitidulus</i> present)	Parameters	Mean	SD	Min	Max	Median
<i>F. polycтена</i>	120 (90, 75.0 %)	volume (l)	529.7	504.2	8.4	2915.4	366.2
		nest density	7.1	5.8	0	24	5
		distance (m)	29.7	32.8	5.9	195.3	16.5
<i>F. rufa</i>	25 (7, 28.0 %)	volume (l)	217.9	264.7	4.7	1169.6	113.1
		nest density	2.5	2.1	0	7	2
		distance (m)	76.0	94.7	10.0	417.9	47.7
<i>F. aquilonia</i>	14 (3, 21.4 %)	volume (l)	514.4	608.6	35.3	2312.2	278.0
		nest density	13.4	0.9	12	14	14
		distance (m)	9.2	8.9	1.1	31.2	5.1
<i>F. pratensis</i>	5 (-, -)	volume (l)	165.7	57.2	108.4	237.5	141.8
		nest density	0.4	0.5	0	1	0
		distance (m)	82.7	65.1	12.2	145.1	122.1
<i>F. lugubris</i>	2 (-, -)	volume (l)	319.6	260.7	135.3	504.0	
		nest density	0.5	0.7	0	1	
		distance (m)	131.6	90.2	67.8	195.3	
Total	166 (100, 60.2 %)						

563

564 **Table 2** Results of the two competing GLIMMIX models showing the effect of red wood ant
 565 host species (*F. polyctena*, *F. rufa*), host nest density (number of nests / 100 m radius of the
 566 focal nest), distance (metres) to nearest nest, and host nest volume (litres) on the occurrence
 567 of *Formicoxenus nitidulus*. Asterisk indicates the better model according to Pearson's and
 568 Spearman's correlation coefficients (results presented under the table).

	DF	F	P
Model 1*			
Host species	1, 76.45	4.87	0.030
Nest density	1, 141	5.47	0.021
Volume (l)	1, 141	4.86	0.029
Model 2			
Host species	1, 72.41	8.24	0.005
Distance (m)	1, 138.1	0.16	0.693
Volume (l)	1, 141	4.77	0.031

*Better model according to both Pearson's and Spearman's correlation coefficients (model 1 predicted occurring probability x nests density: Pearson's $r = 0.65$, $P < 0.0001$, Spearman's $\rho = 0.73$, $P < 0.0001$; model 2 predicted occurring probability x distance: Pearson's $r = -0.27$, $P = 0.0009$, Spearman's $\rho = -0.20$, $P = 0.0167$)

569

570

571 **Fig. 1** *Formicoxenus nitidulus* and *Formica rufa* (Photo by S. K. Härkönen)

572 **Fig. 2** *Formicoxenus nitidulus* a) male, b) regular worker (without ocelli), and c) winged
573 queen (Photos by Veikko Rinne)

574 **Fig. 3** The number of observed *Formica polyctena* (Fpoly) and *F. rufa* (Frufa) nests at
575 varying a) nest volumes (litres), and b) nest densities (number of nests within 100 m radius of
576 the focal nest); the darker bottom sections of the bars indicate the number of nests where
577 *Formicoxenus nitidulus* was found

578 **Fig. 4** Probability of *Formicoxenus nitidulus* occurrence (mean \pm 95 % CL) among red wood
579 ants *Formica polyctena* (black) and *Formica rufa* (grey) at varying a) nest volumes (litres)
580 and b) nest densities (number of nests within 100 metre radius of the focal nest). Original
581 occurrence data (0/1) is also included: triangle pointing up for *F. polyctena* nests and triangle
582 pointing down for *F. rufa* nests