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Comparative analysis of hissing calls in five tit species

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Manuscript Details

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Abstract

Nest predation often leads to breeding failure and is an important component of natural selection that affects the evolution of nest defense behavior in birds. Many tit species give a hissing call as nest defense, but there are few studies of interspecific variation in hissing calls, and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tits (*Parus cinereus*), marsh tits (*Poecile palustris*), varied tits (*Sittiparus varius*), willow tits (*Poecile montanus*), and coal tits (*Periparus ater*) in Saihanba National Forest Park in Hebei and Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the proportion of cinereous, willow, and coal tit individuals giving a hissing call differed significantly but the rate of nest predation was similar. It was also true for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of Liaoning. Cinereous and varied tits showed no differences in clutch size, date of the first egg, nest predation and nesting success between individuals that gave and those that did not give a hissing call. These results indicate that for tit species that breed in nest boxes distributed within the same area, there is interspecific variation in hissing calls but this variation is not significantly correlated with nest predation risk.

Keywords hissing call; nest defense; nest predation; repeatability; Paridae.

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Research Data Related to this Submission

There are no linked research data sets for this submission. The following reason is given:
Data will be made available on request



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Dear Prof. J. J. Bolhuis,

Please find enclosed a manuscript entitled “**Comparative analysis of hissing calls in five tit species**” for possible publication in *Behavioral Processes*.

Many tit species give a hissing call for nest defense, but there are few studies on inter-specific variations in hissing calls and whether these are related to nest predation and nesting success. In this study, we compared the hissing calls of five tit species including cinereous tit (*Parus cinereus*), marsh tit (*Poecile palustris*), varied tit (*Sittiparus varius*), willow tit (*Poecile montanus*), and coal tit (*Periparus ater*) in China.

The main finding of this study is that **for tit species that breed in nest boxes distributed within the same area, there are inter-specific variations in hissing call but these are not significantly correlated with nest predation risk and nesting success.**

We believe that these findings will be of interest to a wide range of researchers, who constitute the readership of your prestigious journal.

This manuscript has not been published or presented elsewhere in part or in entirety, and it is not under consideration for publication by another journal. We have read and understood your journal's policies, and we believe that neither the manuscript nor the study violates any of these. There are no competing interest to declare.

Thank you for your consideration. I am looking forward to hearing from you.

On behalf of the authors, sincerely yours,

Dongmei Wan and Wei Liang

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Response to comments (Ref: BEPROC_2019_100R3)

**Dear Prof. Bolhuis,
Editor-in-Chief,
Behavioural Processes**

**Thank you very much for kindly permitting us to revise our manuscript.
We have now read the comments carefully and have revised the paper as
suggested by you and the reviewers.**

Please find explained in blue bold font below how we have addressed the points.

Thank you very much in advance for your editorial assistance.

Yours sincerely, on behalf of the co-authors,

Wei Liang

Ref: BEPROC_2019_100_R3

Title: Comparative analysis of hissing calls in five tit species

Journal: Behavioural Processes

Dear Dr. Liang,

Thank you for submitting your manuscript to Behavioural Processes. Reviewers have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, please submit your revision within 60 days of accepting to revise. A summary is appended below. While revising the paper please consider the reviewers' comments carefully. We look forward to receiving your detailed response and your revised manuscript.

Reviewer 2 has some final issues that you should address. As soon as I receive your revision I will accept it.

I look forward to receiving your revised manuscript as soon as possible.

Kind regards,

Professor. Bolhuis
Editor-in-Chief
Behavioural Processes

Comments from the editors and reviewers:

-Reviewer 1

- I focused mainly on previous problems/ suggestions. Well solved.

Thank you very much for your helpful comments. We appreciate your time and your kind help.

-Reviewer 2

The revisions the authors made are strong – the paper reads really well! I have only a few minor wording suggestions:

Thank you very much for taking your time and your helpful comments.

Highlights: Given the changes made, I suggest replacing “these are related” in both highlights with “this variation is related”

Revised as you kindly suggested.

Discussion:

Line 235-236: I suggest “there were few snakes and few Swinhoe’s . . .”

Revised. Thanks.

Line 252: I suggest replacing “female birds (Koosa and Tilgar 2016). For example, females” with “female great tits (Koosa and Tilgar 2016) in that females”

Revised.

A nice study!

Thanks. We really appreciate all helpful suggestions from the Editor and Reviewers.

Highlights

Many tit species in the Paridae family give a hissing call for nest defense, but there are few studies on inter-specific variation in hissing calls and whether this variation is related to nest predation and nesting success.

This study showed that there is inter-specific variation in hissing calls of five tit species in China that breed in nest boxes distributed within the same area, but this variation is not significantly correlated with nest predation risk and nesting success.

1 **Comparative analysis of hissing calls in five tit species**

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21 **Running headline:** Hissing calls in tit species

23 **Abstract**

24 Nest predation often leads to breeding failure and is an important component of
25 natural selection that affects the evolution of nest defense behavior in birds.
26 Many tit species give a hissing call as nest defense, but there are few studies of
27 interspecific variation in hissing calls, and whether these are related to nest
28 predation and nesting success. In this study, we compared the hissing calls of
29 five tit species including cinereous tits (*Parus cinereus*), marsh tits (*Poecile*
30 *palustris*), varied tits (*Sittiparus varius*), willow tits (*Poecile montanus*), and
31 coal tits (*Periparus ater*) in Saihanba National Forest Park in Hebei and
32 Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the
33 proportion of cinereous, willow, and coal tit individuals giving a hissing call
34 differed significantly but the rate of nest predation was similar. It was also true
35 for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of
36 Liaoning. Cinereous and varied tits showed no differences in clutch size, date
37 of the first egg, nest predation and nesting success between individuals that
38 gave and those that did not give a hissing call. These results indicate that for tit
39 species that breed in nest boxes distributed within the same area, there is
40 interspecific variation in hissing calls but this variation is not significantly
41 correlated with nest predation risk.

42

43 **Keywords:** hissing call; nest defense; nest predation; repeatability; Paridae.

45 **Introduction**

46 Nest predation often leads to reproductive failure and is a major selective force
47 that affects nest defense behavior in birds (Ricklefs 1969; Martin 1995;
48 Forstmeier and Weiss 2004; Fontaine and Martin 2006; Lima 2009; Tilgar and
49 Moks 2015; Fu et al. 2016; Guppy et al. 2017). Birds have evolved complex
50 anti-predation strategies to protect their nests and demonstrate specific
51 behaviors when facing predators of different types and risk levels (Lima et al.
52 2005; Yorzinski and Vehrencamp 2009; Krams et al. 2010; Yorzinski and Platt
53 2012; Suzuki 2011, 2015; Daniela et al. 2018; Maziarz et al. 2018). Nest stage,
54 sex, nest type and predator location may also have an effect on anti-predator
55 behaviour of birds (Burger 1992; Ritchison 1993; Crisologo and Bonter 2017).
56 For example, when a blue peacock (*Pavo cristatus*) encounters a raccoon during
57 the day, it calls loudly, stretches its neck, opens its wings, and strikes a flying
58 pose as it approaches the predator. However, at night it will instead remain
59 silent and give a soft hissing call (Yorzinski and Platt 2012).

60 In animals, sound is often used to transmit predator information
61 (Zuberbühler 2009; Fasanella and Fernández 2009; Suzuki 2011, 2014, 2015;
62 Fuong et al. 2014; Townsend et al. 2014). Individuals of the same or different
63 species use this acoustic information to evade predation (Sherman 1977; Pipia
64 et al. 2009; Kitchen et al. 2010; Suzuki 2011, 2015; Gill and Bierema 2013;
65 Townsend and Manser 2013). For example, Japanese tits (*Parus minor*) give
66 different warning sounds to indicate the predator type: the “jar” sound is used to

67 indicate a Japanese rat snake (*Elaphe climacophora*) whereas the “chicka”
68 sound indicates a jungle crow (*Corvus macrorhynchos*) (Suzuki 2014). When
69 chicks close to fledging hear the “jar” sound coming from the female they jump
70 out of the nest to evade predation by the snake, but upon hearing the “chicka”
71 sound they instead huddle in the nest to avoid being preyed upon (Suzuki 2011).

72 Some birds have also evolved acoustic Batesian mimicry that simulates the
73 sound of a toxic, inedible, or more dangerous species so as to gain security
74 benefits (Gaul 1952; Sibley 1955; Klump and Shalter 1984; Apel and Weise
75 1986; Rowe et al. 1986; Owings et al. 2002; Kelly et al. 2008; Zub et al 2017).
76 When a female bird like a great tit (*Parus major*) senses the presence of a
77 predator, the bird stretches its wings forward and down rapidly in a curve, raises
78 and extends its tail, and gives a spontaneous hissing call (Perrins 1979; Cramp
79 and Perrins 1994) that leads to its misidentification as a snake by the predator,
80 which is then discouraged from approaching (Cox 1930; Sibley 1955; Rowe et
81 al. 1986; Perrins 1979; Krams et al. 2014). As an additional example, the
82 hissing call of burrowing owls (*Athene cunicularia*) simulates the crackling
83 sound of alerted Prairie rattlesnakes (*Crotalus viridis*) and is used to scare off its
84 predator, the California ground squirrel (*Spermophilus beecheyi*) (Rowe et al.
85 1986).

86 Hissing calls are common in cavity breeding birds including tits (Odum
87 1942; Hinde 1952; Sibley 1955; Apel and Weise 1986; Broughton 2005, 2012).
88 In many tit species, females incubate the eggs while males scarcely engage in

89 alerting behavior or assist with nest defense (Perrins 1979). A recent study
90 showed that hissing females of great tits survive better than silent females
91 (Krams et al. 2014). Playing recordings of the hissing calls of the great tit,
92 Eurasian blue tit (*Cyanistes caeruleus*), and marsh tit (*Poecile palustris*) could
93 lead to lower levels of nest exploration by their predators, the yellow-necked
94 mouse (*Apodemus flavicollis*) (Zub et al. 2017). Among different tit species
95 breeding in the same area, rates of nest predation are lower for those that exhibit
96 hissing behavior than for those that do not (Walankiewicz 2002; Wesołowski
97 2002; Czeszczewik 2004; Wesołowski and Rowiński 2012; Maziarz et al. 2016).
98 However, hissing calls of many tit species have not yet been investigated.
99 Moreover, there is little information on whether there is interspecific variation
100 in hissing calls of tit species located in the same area, and whether the hissing
101 call of different species is related to their life history traits.

102 To address these questions, in this study we compared the hissing calls of
103 five tit species located in the same area. For sympatrically breeding cinereous
104 and varied tits, we also investigated whether there were differences in the
105 breeding parameters of individuals with or without a hissing call, such as date of
106 the first egg, clutch size, nest predation rate and nesting success in order to
107 determine the relationship between hissing call and breeding performance.

108

109 **Materials and methods**

110 **Study area and study species**

111 The Saihanba National Forest Park (SHB) is located in Weichang, 240 km from
112 Chengde City, Hebei Province (42°02'–42°36' N, 116°51'–117°39' E) at an
113 altitude of 1,350–1,650 m. The park has a semi-arid/semi-humid cold-
114 temperature continental monsoon climate and is the main natural secondary
115 forest and plantation forest area in Hebei. Within the park there are plateaus,
116 mountains, forests, and grasslands (Fig. 1; Liu et al. 2017). In 2017, 195
117 wooden nestboxes were put up at a height of 3–4 m on Mongolia scotch pines
118 (*Pinus sylvestris var. mongolica*) and telegraph poles. These nestboxes have an
119 entrance-hole diameter of 4.5 cm and a large chamber (14 cm × 17 cm × 34 cm).

120 The Xianrendong National Nature Reserve (XRD) is located in Zhuanghe,
121 Liaoning Province (39°54'–40°03' N, 122°53'–123°03' E) at an altitude of 200–
122 600 m. It is adjacent to the Yellow Sea and is located in a warm, temperate,
123 humid monsoon climate zone (Fig. 1; Du et al. 2010). A total of 332 and 426
124 wooden nestboxes were put up in 2016 and 2018, with a height of 1.3–4.4 m in
125 pine trees (*Pinus densiflora*), poplar trees (*Alnus japonica*) and Chinese ashes
126 (*Pterocarya stenoptera*). These nestboxes have an entrance-hole diameter of
127 3.5–4.5 cm and a large chamber (outside 14 cm × 16 cm × 34 cm, inside 10 cm
128 × 12 cm × 32 cm).

129 Tits belong to the Paridae family, which comprises small passerine birds
130 that are mainly distributed in the Northern hemisphere and Africa. These small,
131 stocky, woodland species have short bills and a length of 10–22 cm (Gosler and
132 Peter, 2007). Great tits, which were originally distributed in Eurasia, are now

133 classified as three separate species: great tits from Europe to Northwestern Asia,
134 the cinereous tit (*Parus cinereus*) of South Asia, and the Japanese tit of East
135 Asia (Päckert et al. 2005).

136 In 2018, the birds attracted to artificial nest boxes hung in SHB were
137 mainly cinereous tits, willow tits (*Poecile montanus*), and coal tits (*Periparus*
138 *ater*) (Fig. 1, A-C). Between 2016 and 2018, the birds that were attracted to nest
139 boxes hung in XRD were mainly cinereous, marsh, and varied tits (*Sittiparus*
140 *varius*) (Fig. 1, D-F).

141

142 **Field data collection**

143 The nest boxes, particularly those used by tits, were routinely examined during
144 the breeding season. In SHB, we performed our first nest inspection on May 10,
145 2018, and the last nest inspection on August 2, 2018. In XRD, in 2016 our first
146 nest inspection was conducted on April 10, and the last nest inspection was
147 conducted on July 3; in 2018 our first nest inspection was performed on April 8,
148 and our last nest inspection was performed on July 1. During a day, we checked
149 nest boxes between 09:30 a.m. and 16:00 p.m. Hatching status was determined
150 according to clutch size and date of the first egg. The date at which the female
151 laid the last egg was defined as day 0 of the incubation period. In this study, the
152 incubation period of each of the five tit species was approximately 12 days. We
153 divided the incubation period into three stages: early, mid and late incubation.
154 The nest boxes were inspected once during each stage. When we opened the lid

155 of the nest box during inspection, some tits gave a hissing call instead of
156 escaping from the nest. Depending on the response of the female upon opening
157 the nest box, we divided birds into those with or without hissing calls. During
158 field work, we found that the hissing call of the five tit species was highly
159 repeatable—i.e., individuals that did not give a hissing call at the start of the
160 study also did not give any hissing calls later during the breeding period.

161 Nest predation rate was defined as the proportion of depredated nests to the
162 total nests monitored (Krams et al. 2014). Nesting success was defined as the
163 proportion of successful nests (success to fledge at least one young), and it was
164 a dichotomous variable for measuring predation intensity (Pribil 1998).

165

166 **Statistical analysis**

167 Statistical analysis was performed using SPSS v.16.0 for Windows (IBM,
168 Armonk, NY, USA). The one-sample Kolmogorov-Smirnov test was used to
169 analyze the normality of the data. When the data normality condition was met,
170 the t-test or one-way analysis of variance was used to compare mean values.

171 Otherwise, non-parametric tests—i.e., the Mann-Whitney U test and Kruskal-
172 Wallis test—were used. All tests were two-tailed, with a significance level of P
173 < 0.05 . Data are expressed as mean \pm standard deviation (mean \pm SD).

174

175 **Results**

176 During 2018 and 2019 in SHB, 19 out of 32 (59.4%) cinereous tit, 14 out of 17
177 (82.3%) coal tit, and 4 out of 17 (23.5%) willow tit nests contained females that
178 gave hissing calls. There were significant differences among hissing calls of the
179 three tit species located in the same area (Fig. 2; $P = 0.002$, Fisher's exact test).

180 During 2018 in XRD, 16 out of 39 (41.0%) varied tits, 24 out of 40 (60.0%)
181 cinereous tits, and 3 out of 17 (17.6%) marsh tits gave hissing calls. Significant
182 differences were observed in the frequency of hissing calls of the three tit
183 species located in the same area (Fig. 2; $P = 0.011$, Fisher's exact test).

184 In SHB, none of the 66 cinereous tits and 45 coal tits and only one of the
185 31 willow tit nests were depredated. There were no significant differences in
186 predation rates among the three tit species ($P > 0.05$, Fisher's exact test).

187 In XRD, 18 out of 39 (46.2%) varied tits, 14 out of 40 (35.0%) cinereous
188 tits, and 8 out of 19 (42.1%) marsh tit nests were targeted by predators. There
189 were no significant differences in predation rates among the three tit species (P
190 $= 0.597$, Fisher's exact test). For a total of 51 depredated nests, 30 nests were
191 confirmed to be depredated by mice (10%; 3 out of 30 nests) or snakes (90%;
192 including Korean rat-snakes *Elaphe anomala* and steppe rat-snakes *E. dione*).

193 For cinereous tits, 62 tits gave hissing calls whereas 23 tits did not. The
194 date of the first egg and clutch size did not differ significantly between the two
195 groups of tits [9.75 ± 1.31 (n = 60) vs. 9.77 ± 1.23 (n = 22)] ($P = 0.880$, Mann-
196 Whitney U test). Similar rates of nest predation (19.4% vs. 30.4%) and nesting

197 success (64.5% vs. 52.2%) were also observed between the two groups (Fig. 3;
198 nest predation: $P = 0.379$; nesting success: $P = 0.326$, Fisher's exact test).

199 For varied tits, 52 tits gave hissing calls whereas 37 tits did not. The two
200 groups were similar in terms of date of the first egg and clutch size (7.31 ± 1.08
201 vs. 7.10 ± 1.12) ($P = 0.264$, Mann-Whitney U test) as well as nest predation
202 (21.6% vs. 30.8%) and nesting success (56.8% vs. 44.2%) (Fig. 3; nest
203 predation: $P = 0.468$; nesting success: $P = 0.286$, Fisher's exact test).

204

205 **Discussion**

206 Nest predation is a major cause of death in birds and has led to the evolution of
207 morphological, physiological, and behavioral strategies to avoid predation
208 (Lima 2009; Parejo et al. 2013; Fu et al. 2016). Previous work showed that
209 cavity breeding tits give hissing calls to frighten approaching predators (Rowe
210 et al. 1986; Zub et al. 2017) and thereby increase the survival rate of female
211 birds and fledglings (Krams et al. 2014). Our study indicated that in SHB, there
212 were significant differences in the proportions of individuals giving a hissing
213 call among cinereous, willow and marsh tits. Furthermore, in XRD, differences
214 were also observed in the proportions of cinereous, varied, and marsh tits giving
215 hissing calls. However, in both SHB of Hebei and XRD of Liaoning, nest
216 predation rates of tits breeding in nest boxes located in the same area did not
217 differ. Cinereous and varied tits showed no differences in clutch size, date of the

218 first egg, nest predation rate and nesting success between birds with and without
219 hissing calls.

220 Tit species in the wild are known to give hissing calls (Pickens 1928;
221 Sibley 1995). However, there have been few studies investigating interspecific
222 variation in hissing calls of tit species (Sibley 1995; Krams et al. 2014; Koosa
223 and Tilgar 2016; Zub et al. 2017). An early report suggested that the hissing
224 calls of tits were an acoustic form of Batesian mimicry and provided a brief
225 description of the hissing calls of different species (Sibley 1995). Here, we
226 recorded in detail the hissing calls of five tit species located in the same area
227 and investigated interspecific differences.

228 We found that 60% of individual cinereous tits gave a hissing call; this is
229 comparable to the 70% reported in a previous study in which hissing calls of
230 great tits were found to substantially reduce the predation rate of breeding
231 females (Krams et al. 2014). The hissing call of tits was also shown to
232 discourage the nest exploration behavior of yellow-necked mouse (*Apodemus*
233 *flavicollis*) (Zub et al. 2017). However, hissing call behavior did not reduce the
234 rate of nest predation in tits. In SHB, the artificial nest boxes were mainly
235 distributed in plantations located in areas where there were few snakes and few
236 Swinhoe's striped squirrels (*Tamiops swinhoei*)—two of the natural predators of
237 tits—due to the high altitude and low temperature. On the other hand, in XRD,
238 nest predation rates of cinereous, varied, and marsh tits did not differ despite
239 variation in the proportion of individuals with hissing calls. This is because the

240 main nest predators in this area were Korean and steppe rat-snakes, which are
241 not influenced by the hissing calls of tits.

242 An earlier study found that the nest predation rate is markedly lower for
243 great tits compared to the two species without hissing calls (Krams et al. 2014).
244 A possible reason for the discrepancy between our study and this earlier study is
245 the small sample size (85 cinereous and 89 varied tits vs. 477 samples in the
246 study by Krams et al. 2014). Additionally, the predators in their study were
247 mainly European pine marten (*Martes martes*), least weasel (*Mustela nivalis*),
248 and great spotted woodpecker (*Dendrocopos major*), which are more likely
249 scared off by the snake-like hissing calls of great tits than the predators in our
250 study area (mainly snakes) (Weatherhead and Blouin-Demers 2004).

251 Hissing calls have been reported not to be related to the breeding
252 performance of female great tits (Koosa and Tilgar 2016) in that females with
253 and without hissing calls were found to lay a similar clutch size. Our results
254 support this observation. Furthermore, we also determined that the hissing calls
255 of all five tit species are unrelated to nest predation risk.

256 In conclusion, our results showed that hissing calls are common among
257 sympatrically breeding tit species such as cinereous, willow, coal, marsh, and
258 varied tits. In addition, the hissing calls of cinereous and varied tits were
259 unrelated to nest predation risk or life history traits such as date of the first egg,

260 clutch size and nesting success. The present study made a contribution to intra-
261 and inter-specific differences in vocal behavior in closely related species.

263

264 **Ethics** The experiments reported here comply with the current laws of China.

265 Fieldwork was carried out under the permission from Saihanba National Forest

266 Park and Xianrendong National Nature Reserve, China. Experimental

267 procedures were in agreement with the Animal Research Ethics Committee of

268 Hainan Provincial Education Centre for Ecology and Environment, Hainan

269 Normal University (permit no. HNECEE-2011-001).

270

271 **Data accessibility** Data used in this study are available in Electronic

272 Supplementary Materials.

273

274 **Competing interests** The authors declare that they have no competing interests.

275

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432

433 **Legends to figures**

434

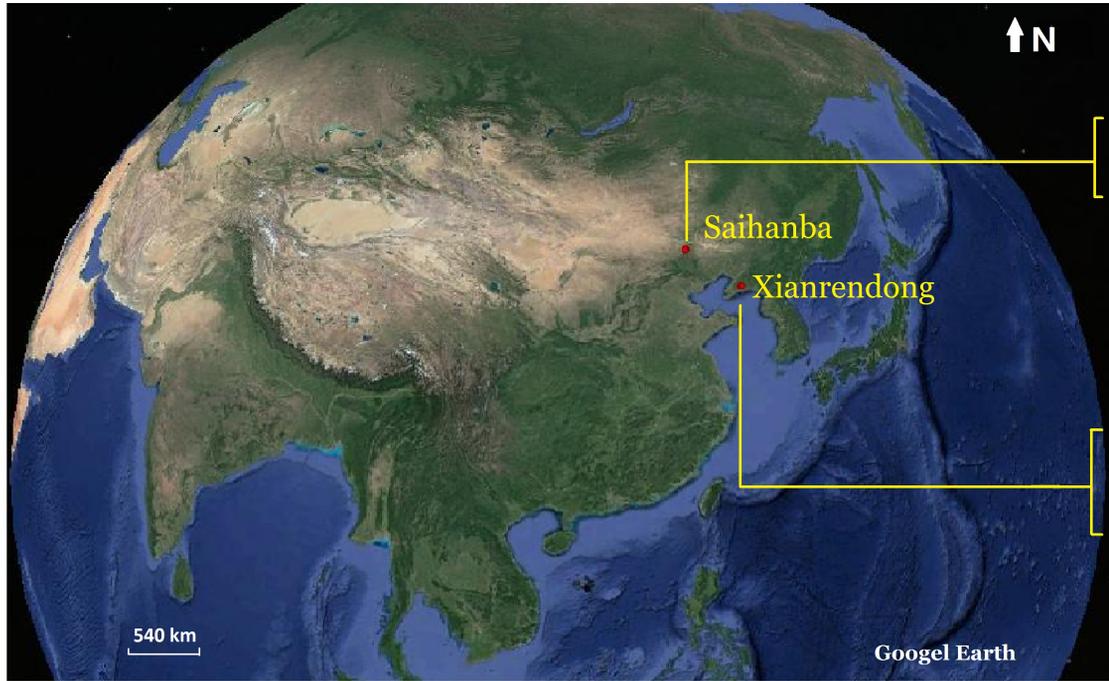
435 **Figure 1.** Study areas and study species in this study. Capital letters (A-F) refer
436 to bird species and lowercase letters (a-f) refer to its nest and eggs. A and D
437 refer to cinereous tit; B coal tit; C willow tit; E varied tit; and F marsh tit.

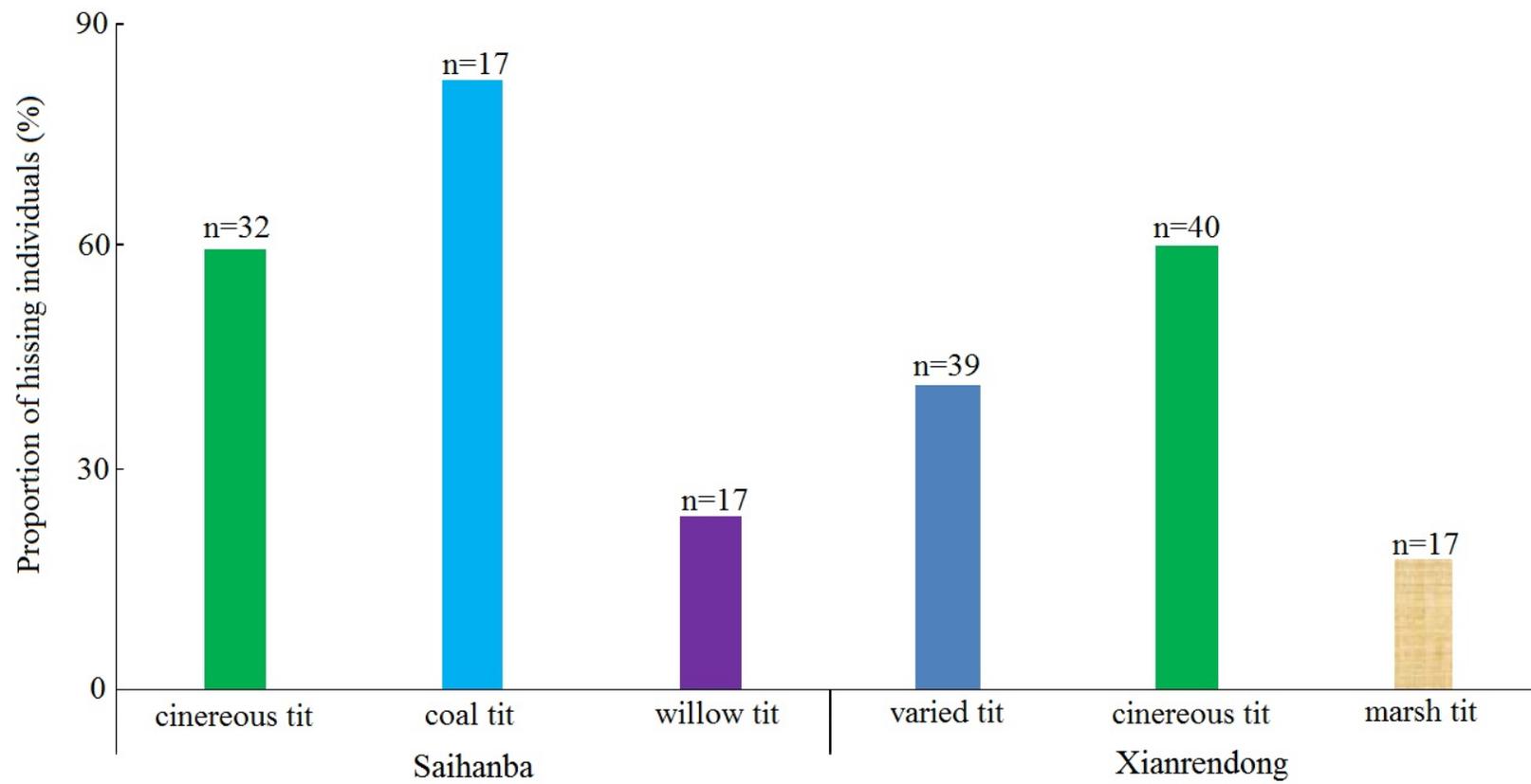
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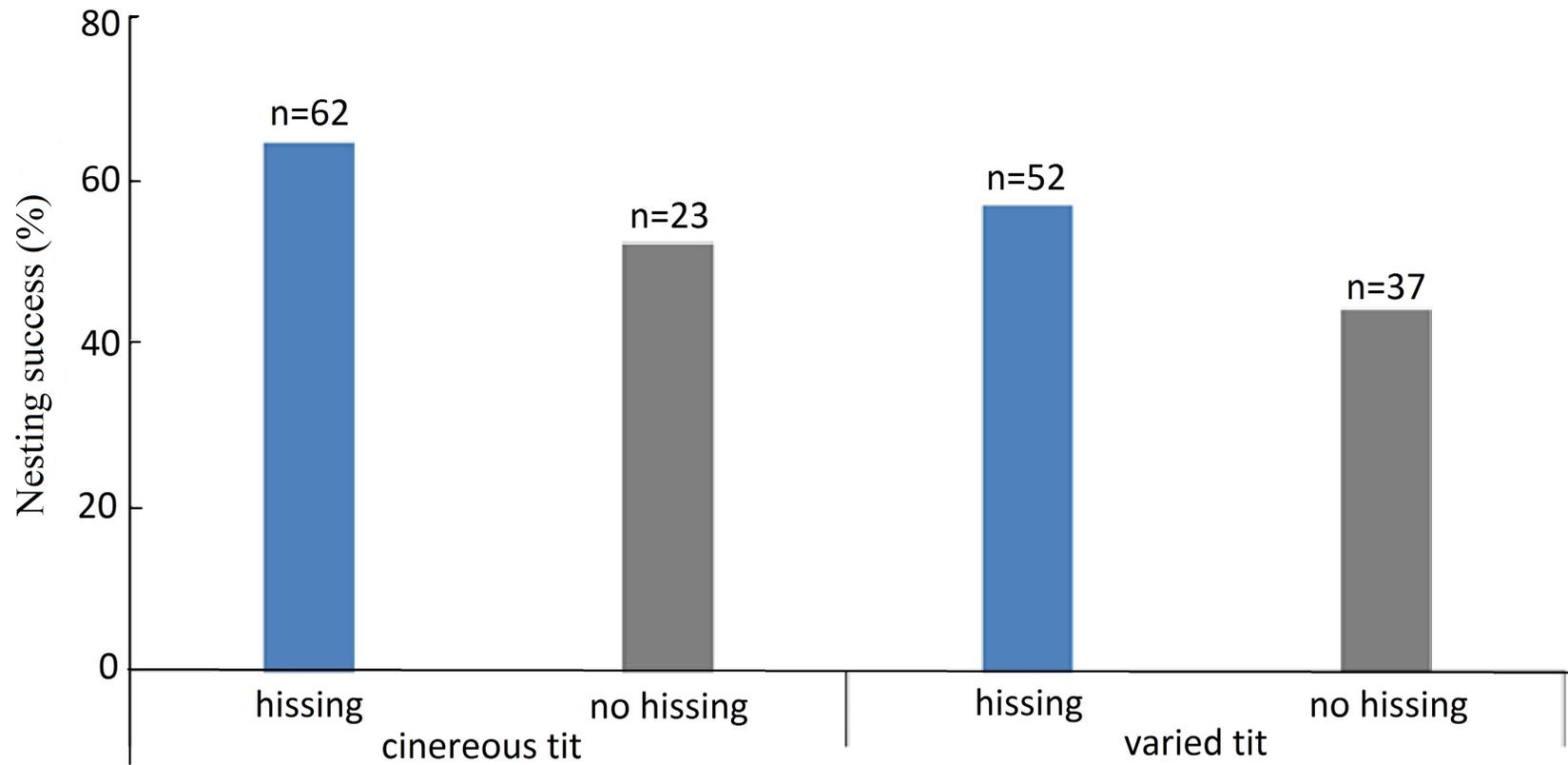
439 **Figure 2.** Proportion of hissing individuals in five tit species at two study sites.

440

441 **Figure 3.** Comparison of nesting success (% eggs that resulted in fledged young)
442 between individuals with and without hissing call in cinereous and varied tits.







CRedit author statement

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1 **Comparative analysis of hissing calls in five tit species**

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21 **Running headline:** Hissing calls in tit species

23 **Abstract**

24 Nest predation often leads to breeding failure and is an important component of
25 natural selection that affects the evolution of nest defense behavior in birds.
26 Many tit species give a hissing call as nest defense, but there are few studies of
27 interspecific variation in hissing calls, and whether these are related to nest
28 predation and nesting success. In this study, we compared the hissing calls of
29 five tit species including cinereous tits (*Parus cinereus*), marsh tits (*Poecile*
30 *palustris*), varied tits (*Sittiparus varius*), willow tits (*Poecile montanus*), and
31 coal tits (*Periparus ater*) in Saihanba National Forest Park in Hebei and
32 Xianrendong National Nature Reserve in Liaoning. In Saihanba of Hebei, the
33 proportion of cinereous, willow, and coal tit individuals giving a hissing call
34 differed significantly but the rate of nest predation was similar. It was also true
35 for the three tit species (cinereous, varied, and marsh tits) in Xianrendong of
36 Liaoning. Cinereous and varied tits showed no differences in clutch size, date
37 of the first egg, nest predation and nesting success between individuals that
38 gave and those that did not give a hissing call. These results indicate that for tit
39 species that breed in nest boxes distributed within the same area, there is
40 interspecific variation in hissing calls but this variation is not significantly
41 correlated with nest predation risk.

42

43 **Keywords:** hissing call; nest defense; nest predation; repeatability; Paridae.

45 **Introduction**

46 Nest predation often leads to reproductive failure and is a major selective force
47 that affects nest defense behavior in birds (Ricklefs 1969; Martin 1995;
48 Forstmeier and Weiss 2004; Fontaine and Martin 2006; Lima 2009; Tilgar and
49 Moks 2015; Fu et al. 2016; Guppy et al. 2017). Birds have evolved complex
50 anti-predation strategies to protect their nests and demonstrate specific
51 behaviors when facing predators of different types and risk levels (Lima et al.
52 2005; Yorzinski and Vehrencamp 2009; Krams et al. 2010; Yorzinski and Platt
53 2012; Suzuki 2011, 2015; Daniela et al. 2018; Maziarz et al. 2018). Nest stage,
54 sex, nest type and predator location may also have an effect on anti-predator
55 behaviour of birds (Burger 1992; Ritchison 1993; Crisologo and Bonter 2017).
56 For example, when a blue peacock (*Pavo cristatus*) encounters a raccoon during
57 the day, it calls loudly, stretches its neck, opens its wings, and strikes a flying
58 pose as it approaches the predator. However, at night it will instead remain
59 silent and give a soft hissing call (Yorzinski and Platt 2012).

60 In animals, sound is often used to transmit predator information
61 (Zuberbühler 2009; Fasanella and Fernández 2009; Suzuki 2011, 2014, 2015;
62 Fuong et al. 2014; Townsend et al. 2014). Individuals of the same or different
63 species use this acoustic information to evade predation (Sherman 1977; Pipia
64 et al. 2009; Kitchen et al. 2010; Suzuki 2011, 2015; Gill and Bierema 2013;
65 Townsend and Manser 2013). For example, Japanese tits (*Parus minor*) give
66 different warning sounds to indicate the predator type: the “jar” sound is used to

67 indicate a Japanese rat snake (*Elaphe climacophora*) whereas the “chicka”
68 sound indicates a jungle crow (*Corvus macrorhynchos*) (Suzuki 2014). When
69 chicks close to fledging hear the “jar” sound coming from the female they jump
70 out of the nest to evade predation by the snake, but upon hearing the “chicka”
71 sound they instead huddle in the nest to avoid being preyed upon (Suzuki 2011).

72 Some birds have also evolved acoustic Batesian mimicry that simulates the
73 sound of a toxic, inedible, or more dangerous species so as to gain security
74 benefits (Gaul 1952; Sibley 1955; Klump and Shalter 1984; Apel and Weise
75 1986; Rowe et al. 1986; Owings et al. 2002; Kelly et al. 2008; Zub et al 2017).
76 When a female bird like a great tit (*Parus major*) senses the presence of a
77 predator, the bird stretches its wings forward and down rapidly in a curve, raises
78 and extends its tail, and gives a spontaneous hissing call (Perrins 1979; Cramp
79 and Perrins 1994) that leads to its misidentification as a snake by the predator,
80 which is then discouraged from approaching (Cox 1930; Sibley 1955; Rowe et
81 al. 1986; Perrins 1979; Krams et al. 2014). As an additional example, the
82 hissing call of burrowing owls (*Athene cunicularia*) simulates the crackling
83 sound of alerted Prairie rattlesnakes (*Crotalus viridis*) and is used to scare off its
84 predator, the California ground squirrel (*Spermophilus beecheyi*) (Rowe et al.
85 1986).

86 Hissing calls are common in cavity breeding birds including tits (Odum
87 1942; Hinde 1952; Sibley 1955; Apel and Weise 1986; Broughton 2005, 2012).
88 In many tit species, females incubate the eggs while males scarcely engage in

89 alerting behavior or assist with nest defense (Perrins 1979). A recent study
90 showed that hissing females of great tits survive better than silent females
91 (Krams et al. 2014). Playing recordings of the hissing calls of the great tit,
92 Eurasian blue tit (*Cyanistes caeruleus*), and marsh tit (*Poecile palustris*) could
93 lead to lower levels of nest exploration by their predators, the yellow-necked
94 mouse (*Apodemus flavicollis*) (Zub et al. 2017). Among different tit species
95 breeding in the same area, rates of nest predation are lower for those that exhibit
96 hissing behavior than for those that do not (Walankiewicz 2002; Wesołowski
97 2002; Czeszczewik 2004; Wesołowski and Rowiński 2012; Maziarz et al. 2016).
98 However, hissing calls of many tit species have not yet been investigated.
99 Moreover, there is little information on whether there is interspecific variation
100 in hissing calls of tit species located in the same area, and whether the hissing
101 call of different species is related to their life history traits.

102 To address these questions, in this study we compared the hissing calls of
103 five tit species located in the same area. For sympatrically breeding cinereous
104 and varied tits, we also investigated whether there were differences in the
105 breeding parameters of individuals with or without a hissing call, such as date of
106 the first egg, clutch size, nest predation rate and nesting success in order to
107 determine the relationship between hissing call and breeding performance.

108

109 **Materials and methods**

110 **Study area and study species**

111 The Saihanba National Forest Park (SHB) is located in Weichang, 240 km from
112 Chengde City, Hebei Province (42°02'–42°36' N, 116°51'–117°39' E) at an
113 altitude of 1,350–1,650 m. The park has a semi-arid/semi-humid cold-
114 temperature continental monsoon climate and is the main natural secondary
115 forest and plantation forest area in Hebei. Within the park there are plateaus,
116 mountains, forests, and grasslands (Fig. 1; Liu et al. 2017). In 2017, 195
117 wooden nestboxes were put up at a height of 3–4 m on Mongolia scotch pines
118 (*Pinus sylvestris var. mongolica*) and telegraph poles. These nestboxes have an
119 entrance-hole diameter of 4.5 cm and a large chamber (14 cm × 17 cm × 34 cm).

120 The Xianrendong National Nature Reserve (XRD) is located in Zhuanghe,
121 Liaoning Province (39°54'–40°03' N, 122°53'–123°03' E) at an altitude of 200–
122 600 m. It is adjacent to the Yellow Sea and is located in a warm, temperate,
123 humid monsoon climate zone (Fig. 1; Du et al. 2010). A total of 332 and 426
124 wooden nestboxes were put up in 2016 and 2018, with a height of 1.3–4.4 m in
125 pine trees (*Pinus densiflora*), poplar trees (*Alnus japonica*) and Chinese ashes
126 (*Pterocarya stenoptera*). These nestboxes have an entrance-hole diameter of
127 3.5–4.5 cm and a large chamber (outside 14 cm × 16 cm × 34 cm, inside 10 cm
128 × 12 cm × 32 cm).

129 Tits belong to the Paridae family, which comprises small passerine birds
130 that are mainly distributed in the Northern hemisphere and Africa. These small,
131 stocky, woodland species have short bills and a length of 10–22 cm (Gosler and
132 Peter, 2007). Great tits, which were originally distributed in Eurasia, are now

133 classified as three separate species: great tits from Europe to Northwestern Asia,
134 the cinereous tit (*Parus cinereus*) of South Asia, and the Japanese tit of East
135 Asia (Päckert et al. 2005).

136 In 2018, the birds attracted to artificial nest boxes hung in SHB were
137 mainly cinereous tits, willow tits (*Poecile montanus*), and coal tits (*Periparus*
138 *ater*) (Fig. 1, A-C). Between 2016 and 2018, the birds that were attracted to nest
139 boxes hung in XRD were mainly cinereous, marsh, and varied tits (*Sittiparus*
140 *varius*) (Fig. 1, D-F).

141

142 **Field data collection**

143 The nest boxes, particularly those used by tits, were routinely examined during
144 the breeding season. In SHB, we performed our first nest inspection on May 10,
145 2018, and the last nest inspection on August 2, 2018. In XRD, in 2016 our first
146 nest inspection was conducted on April 10, and the last nest inspection was
147 conducted on July 3; in 2018 our first nest inspection was performed on April 8,
148 and our last nest inspection was performed on July 1. During a day, we checked
149 nest boxes between 09:30 a.m. and 16:00 p.m. Hatching status was determined
150 according to clutch size and date of the first egg. The date at which the female
151 laid the last egg was defined as day 0 of the incubation period. In this study, the
152 incubation period of each of the five tit species was approximately 12 days. We
153 divided the incubation period into three stages: early, mid and late incubation.
154 The nest boxes were inspected once during each stage. When we opened the lid

155 of the nest box during inspection, some tits gave a hissing call instead of
156 escaping from the nest. Depending on the response of the female upon opening
157 the nest box, we divided birds into those with or without hissing calls. During
158 field work, we found that the hissing call of the five tit species was highly
159 repeatable—i.e., individuals that did not give a hissing call at the start of the
160 study also did not give any hissing calls later during the breeding period.

161 Nest predation rate was defined as the proportion of depredated nests to the
162 total nests monitored (Krams et al. 2014). Nesting success was defined as the
163 proportion of successful nests (success to fledge at least one young), and it was
164 a dichotomous variable for measuring predation intensity (Pribil 1998).

165

166 **Statistical analysis**

167 Statistical analysis was performed using SPSS v.16.0 for Windows (IBM,
168 Armonk, NY, USA). The one-sample Kolmogorov-Smirnov test was used to
169 analyze the normality of the data. When the data normality condition was met,
170 the t-test or one-way analysis of variance was used to compare mean values.

171 Otherwise, non-parametric tests—i.e., the Mann-Whitney U test and Kruskal-
172 Wallis test—were used. All tests were two-tailed, with a significance level of P
173 < 0.05 . Data are expressed as mean \pm standard deviation (mean \pm SD).

174

175 **Results**

176 During 2018 and 2019 in SHB, 19 out of 32 (59.4%) cinereous tit, 14 out of 17
177 (82.3%) coal tit, and 4 out of 17 (23.5%) willow tit nests contained females that
178 gave hissing calls. There were significant differences among hissing calls of the
179 three tit species located in the same area (Fig. 2; $P = 0.002$, Fisher's exact test).

180 During 2018 in XRD, 16 out of 39 (41.0%) varied tits, 24 out of 40 (60.0%)
181 cinereous tits, and 3 out of 17 (17.6%) marsh tits gave hissing calls. Significant
182 differences were observed in the frequency of hissing calls of the three tit
183 species located in the same area (Fig. 2; $P = 0.011$, Fisher's exact test).

184 In SHB, none of the 66 cinereous tits and 45 coal tits and only one of the
185 31 willow tit nests were depredated. There were no significant differences in
186 predation rates among the three tit species ($P > 0.05$, Fisher's exact test).

187 In XRD, 18 out of 39 (46.2%) varied tits, 14 out of 40 (35.0%) cinereous
188 tits, and 8 out of 19 (42.1%) marsh tit nests were targeted by predators. There
189 were no significant differences in predation rates among the three tit species (P
190 $= 0.597$, Fisher's exact test). For a total of 51 depredated nests, 30 nests were
191 confirmed to be depredated by mice (10%; 3 out of 30 nests) or snakes (90%;
192 including Korean rat-snakes *Elaphe anomala* and steppe rat-snakes *E. dione*).

193 For cinereous tits, 62 tits gave hissing calls whereas 23 tits did not. The
194 date of the first egg and clutch size did not differ significantly between the two
195 groups of tits [9.75 ± 1.31 (n = 60) vs. 9.77 ± 1.23 (n = 22)] ($P = 0.880$, Mann-
196 Whitney U test). Similar rates of nest predation (19.4% vs. 30.4%) and nesting

197 success (64.5% vs. 52.2%) were also observed between the two groups (Fig. 3;
198 nest predation: $P = 0.379$; nesting success: $P = 0.326$, Fisher's exact test).

199 For varied tits, 52 tits gave hissing calls whereas 37 tits did not. The two
200 groups were similar in terms of date of the first egg and clutch size (7.31 ± 1.08
201 vs. 7.10 ± 1.12) ($P = 0.264$, Mann-Whitney U test) as well as nest predation
202 (21.6% vs. 30.8%) and nesting success (56.8% vs. 44.2%) (Fig. 3; nest
203 predation: $P = 0.468$; nesting success: $P = 0.286$, Fisher's exact test).

204

205 Discussion

206 Nest predation is a major cause of death in birds and has led to the evolution of
207 morphological, physiological, and behavioral strategies to avoid predation
208 (Lima 2009; Parejo et al. 2013; Fu et al. 2016). Previous work showed that
209 cavity breeding tits give hissing calls to frighten approaching predators (Rowe
210 et al. 1986; Zub et al. 2017) and thereby increase the survival rate of female
211 birds and fledglings (Krams et al. 2014). Our study indicated that in SHB, there
212 were significant differences in the proportions of individuals giving a hissing
213 call among cinereous, willow and marsh tits. Furthermore, in XRD, differences
214 were also observed in the proportions of cinereous, varied, and marsh tits giving
215 hissing calls. However, in both SHB of Hebei and XRD of Liaoning, nest
216 predation rates of tits breeding in nest boxes located in the same area did not
217 differ. Cinereous and varied tits showed no differences in clutch size, date of the

218 first egg, nest predation rate and nesting success between birds with and without
219 hissing calls.

220 Tit species in the wild are known to give hissing calls (Pickens 1928;
221 Sibley 1995). However, there have been few studies investigating interspecific
222 variation in hissing calls of tit species (Sibley 1995; Krams et al. 2014; Koosa
223 and Tilgar 2016; Zub et al. 2017). An early report suggested that the hissing
224 calls of tits were an acoustic form of Batesian mimicry and provided a brief
225 description of the hissing calls of different species (Sibley 1995). Here, we
226 recorded in detail the hissing calls of five tit species located in the same area
227 and investigated interspecific differences.

228 We found that 60% of individual cinereous tits gave a hissing call; this is
229 comparable to the 70% reported in a previous study in which hissing calls of
230 great tits were found to substantially reduce the predation rate of breeding
231 females (Krams et al. 2014). The hissing call of tits was also shown to
232 discourage the nest exploration behavior of yellow-necked mouse (*Apodemus*
233 *flavicollis*) (Zub et al. 2017). However, hissing call behavior did not reduce the
234 rate of nest predation in tits. In SHB, the artificial nest boxes were mainly
235 distributed in plantations located in areas where there were few snakes and few
236 Swinhoe's striped squirrels (*Tamiops swinhoei*)—two of the natural predators of
237 tits—due to the high altitude and low temperature. On the other hand, in XRD,
238 nest predation rates of cinereous, varied, and marsh tits did not differ despite
239 variation in the proportion of individuals with hissing calls. This is because the

240 main nest predators in this area were Korean and steppe rat-snakes, which are
241 not influenced by the hissing calls of tits.

242 An earlier study found that the nest predation rate is markedly lower for
243 great tits compared to the two species without hissing calls (Krams et al. 2014).
244 A possible reason for the discrepancy between our study and this earlier study is
245 the small sample size (85 cinereous and 89 varied tits vs. 477 samples in the
246 study by Krams et al. 2014). Additionally, the predators in their study were
247 mainly European pine marten (*Martes martes*), least weasel (*Mustela nivalis*),
248 and great spotted woodpecker (*Dendrocopos major*), which are more likely
249 scared off by the snake-like hissing calls of great tits than the predators in our
250 study area (mainly snakes) (Weatherhead and Blouin-Demers 2004).

251 Hissing calls have been reported not to be related to the breeding
252 performance of female great titsbirds (Koosa and Tilgar 2016) in that ~~For~~
253 ~~example~~, females with and without hissing calls were found to lay a similar
254 clutch size. Our results support this observation. Furthermore, we also
255 determined that the hissing calls of all five tit species are unrelated to nest
256 predation risk.

257 In conclusion, our results showed that hissing calls are common among
258 sympatrically breeding tit species such as cinereous, willow, coal, marsh, and
259 varied tits. In addition, the hissing calls of cinereous and varied tits were
260 unrelated to nest predation risk or life history traits such as date of the first egg,

261 clutch size and nesting success. The present study made a contribution to intra-
262 and inter-specific differences in vocal behavior in closely related species.

264

265 **Ethics** The experiments reported here comply with the current laws of China.

266 Fieldwork was carried out under the permission from Saihanba National Forest

267 Park and Xianrendong National Nature Reserve, China. Experimental

268 procedures were in agreement with the Animal Research Ethics Committee of

269 Hainan Provincial Education Centre for Ecology and Environment, Hainan

270 Normal University (permit no. HNECEE-2011-001).

271

272 **Data accessibility** Data used in this study are available in Electronic

273 Supplementary Materials.

274

275 **Competing interests** The authors declare that they have no competing interests.

276

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279

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433

434 **Legends to figures**

435

436 **Figure 1.** Study areas and study species in this study. Capital letters (A-F) refer
437 to bird species and lowercase letters (a-f) refer to its nest and eggs. A and D
438 refer to cinereous tit; B coal tit; C willow tit; E varied tit; and F marsh tit.

439

440 **Figure 2.** Proportion of hissing individuals in five tit species at two study sites.

441

442 **Figure 3.** Comparison of nesting success (% eggs that resulted in fledged young)
443 between individuals with and without hissing call in cinereous and varied tits.

Highlights

Many tit species in the Paridae family give a hissing call for nest defense, but there are few studies on inter-specific variation in hissing calls and whether this variation is ~~these are~~ related to nest predation and nesting success.

This study showed that there is inter-specific variation in hissing calls of five tit species in China that breed in nest boxes distributed within the same area, but this variation ~~these is~~ ~~are~~ not significantly correlated with nest predation risk and nesting success.