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# Interspecific differences of stridulatory signals in three species of bark beetles from the genus *Polygraphus* Er. (Coleoptera: Curculionidae, Scolytinae) inhabiting the island of Sakhalin

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Stridulatory signals are involved in conspecific interactions between bark beetles (Coleoptera: Curculionidae, Scolytinae). In this study, we compared the qualitative profiles of acoustic signals in three species from the genus *Polygraphus* Er. Sympatry can be periodically observed in two of them – *P. proximus* and *P. subopacus*. Sporadically they occur on the same plants. *P. nigrielytris* colonize distinctly different host plant species; however, on the island of Sakhalin it inhabits the same biotopes. The purpose of the study is to identify species-specific parameters and the extent of differences in stridulatory signals of these species. Airborne signals produced during the contact of males of the same species were experimentally recorded. Among tested parameters of stridulatory signals, as the most species-specific were noted: chirp duration, number of tooth-strikes per chirp, and intertooth-strike interval.

1 **Interspecific differences of stridulatory signals in**  
2 **three species of bark beetles from the genus**  
3 ***Polygraphus* Er. (Coleoptera: Curculionidae,**  
4 **Scolytinae) inhabiting the island of Sakhalin**

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18

19 **Abstract**

20 Stridulatory signals are involved in conspecific interactions between bark beetles  
21 (Coleoptera: Curculionidae, Scolytinae). In this study, we compared the qualitative profiles of  
22 acoustic signals in three species from the genus *Polygraphus* Er. Sympatry can be periodically  
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27 produced during the contact of males of the same species were experimentally recorded. Among  
28 tested parameters of stridulatory signals, as the most species-specific were noted: chirp duration,  
29 number of tooth-strikes per chirp, and intertooth-strike interval.

30

31 **Introduction**

32 Airborne sounds and solid-borne vibrations are widely used by animals as  
33 communication signals (Dumortier, 1963; Greenfield, 2002; Cocroft & Rodriguez, 2005).  
34 According to one of the latest generalized assessments vibrational signals, are used as a

35 communication channel by 92% of all described insect species (Cocroft & Rodriguez, 2005).  
36 Numerous studies on this type of communication analyze variability of inter and intra species  
37 signals among grasshoppers, crickets, and cicadas (Gerhardt & Huber, 2002; Greenfield, 2002,  
38 2016; Boulard, 2005; Heller, 2005; Henry, 2005; Hoikkala, 2005; Sueur, 2005; Stewart &  
39 Sandberg, 2005). A group of leading researchers investigating different aspects of the  
40 transmission and reception of vibration signals proposed a conception of new term  
41 “semiophysicals” (Mazzoni et al., 2018) for vibrational signals to underline their similarity, in  
42 terms of functions, with semiochemicals (Blum, 1996). It is worth taking into account that there  
43 are bimodal signals that cause an extremely weak response from the recipient during the  
44 broadcast separately of airborne component of signal or substrate borne vibration. However, they  
45 may have a high synergistic effect during reception in complicity (e.g. red-eyed treefrog  
46 *Agalychnis callidryas* Cope., (Kiser et al., 2018). Thus, “semiophysicals” could be a  
47 generalization for signals of physical origin transmitting via gas and solid substrate, but it still  
48 has not clear definition.

49         It should be noted that the mechanisms of sound production and reception are also  
50 widespread among Hymenoptera, Hemiptera, and Coleoptera, which remain poorly studied in  
51 this regard despite their predominate species diversity (Kojima et al, 2012; Breidbach, 1986;  
52 Wessel, 2006). Insects that live both on the surface and inside plants are of particular interest  
53 since plants are good mediators of vibrational signals (Michelsen et al., 1982; McVean & Field,  
54 1996).

55         Bark beetles (Coleoptera: Scolytinae) produce signals using stridulation – a method of  
56 producing sounds by rubbing of the scarperlike structures “*plectrum*” against a special filelike  
57 series of ridges “*pars stridens*” (Barr, 1969). These signals are used in conspecific interactions  
58 (Barr, 1969; Ryker & Rudinsky 1976; Yturalde & Hofstetter, 2015). It has been suggested that  
59 *Ips pini* Say females stridulation signals may have potential for protection against predators  
60 Lewis & Cane, 1990) but this hypothesis has not been confirmed in feather studies (Sivalinghem,  
61 2011). It is still not clear which of the signals, an airborne signal or a solid-borne one, is  
62 perceived by this insects (Fleming et al., 2013; Dobai et al., 2017). Due to dense population of  
63 bark beetles, weak signals possibly can be perceived by the receiver via one of the two channels,  
64 or both simultaneously (Fleming et al., 2013).

65 Almost all Holarctic representatives of the genus *Polygraphus* Er. occur on Pinaceae  
66 (Krivolutskaya 1996; Nobuchi, 1979; Wood and Bright, 1992), for example, the fir bark beetle *P*  
67 *subopacus* is specific to *Picea* spp., and the four-eyed fir bark beetle *Polygraphus proximus*  
68 attacks almost exclusively *Abies* Mill. trees (Wood and Bright, 1992). However, there are  
69 exceptions, for example, *P. nigrielytris* can be found exclusively on Angiosperms, *Sorbus* L. and  
70 *Alnus* Mill. in particular (Krivolutskaya 1996).

71 In this study, we compared the qualitative profiles of acoustic signals of three species,  
72 and two of these, *P. proximus* and *P. subopacus*, can be sporadically found on the same host  
73 plant. The third species, *P. nigrielytris*, is distinctly different from the former two species  
74 according to host-plant specialization (Krivolutskaya, 1958). The purpose of the study is to  
75 reveal the variants and the degree of differences in stridulatory signals required for interspecific  
76 differentiation of bark beetles within the genus *Polygraphus*, which are allopatric and sympatric  
77 with regard to the host plant.

78

## 79 **Materials & Methods**

### 80 **Collection and storage of insects**

81 Imagoes of three tested bark beetle species were collected from the brood trees *P.*  
82 *proximus* on *Abies sahalinensis*, *P. subopacus* on *Larix gmelinii* and *P. nigrielytris* on *Sorbus*  
83 *commixta* in May 2018 on the island of Sakhalin in the territory of Krasnogorsky State Nature  
84 Reserve (48°29'22,2" N, 142°1'49,7" W). Species and sexual identification was performed based  
85 on morphological characteristics (Stark 1952, Krivolutskaya, 1958). Unmated insects were  
86 placed individually in separate marked 5 ml glass tubes with a moistened cotton plug and were  
87 stored before recording procedure at 4 °C for one day.

### 88 **Morphological measurements**

89 An image of a longitudinal section of the imago was generated using an X-ray  
90 microtomography device XWT 160-TC (X-RAY WorX; Garbsen; Germany) at Tomsk  
91 Polytechnic University (Fig. 1a). The images of the elytron-tergite stridulatory apparatus of  
92 males were prepared using a Tabletop Hitachi (Tokyo, Japan) 3000 TM scanning electron  
93 microscope (Fig. 2 b, c) at Tomsk State University. Morphological characteristics such as pars  
94 stridens and number of ridges on it (Yturralde and Hofstetter. 2015; Kerchev, 2018) were

95 measured using Levenhuk ToupView software (release date – 10/15/2015; Levenhuk LabZZ,  
96 Tampa, United States).

97

98

### Fig 1.

99

#### Design

100 Male-male interactions were recorded inside the arena (diameter of 1 cm), a tube with a  
101 microphone installed inside (Kerchev, 2018), no individual was used for trial twice. For each  
102 species were prepared recordings of 30 pairs of beetles. For further analysis, we selected 60 (20  
103 per species) files containing clear distinguishable signals and fewer noises.

104 Audio recording was performed using a Behringer condenser microphone (Willich-  
105 Mьnchheide II, Germany) (model: ECM 8000; 15–20 000 Hz), and a Zoom R16 digital recorder  
106 (Tokyo, Japan); frequency range: 20 Hz – 44.1 kHz; sampling rate: 24-bit). The recorded signals  
107 for a duration of 10-15 minutes were saved in the WAV format. Recording was carried out in the  
108 Krasnogorsk forestry office (Krasnogorsk, Sakhalin region) in a 3 L semi-anechoic chamber  
109 covered with an echo-absorbing coating (2 cm wave). The microphone positioned inside the  
110 arena (glass tube diameter of 1 cm). During recording procedure the membrane of the  
111 microphone was at a distance of 1.5 cm directly above the beetles (Kerchev, 2018). The recorded  
112 signals were analyzed in the Laboratory of Monitoring of Forest Ecosystems, IMCES SB RAS  
113 (Tomsk).

114

#### Terminology and measurements

115 For each recording, the indicators analyzed were syllable duration, number of chirps per  
116 syllable, chirp rate, chirp duration, interchirp interval, number of tooth-strikes per chirp, and  
117 intertooth-strikes interval according to the terminology proposed in previous studies (Pureswaran  
118 et al. 2016; Kerchev, 2018). Individual chirps were identified with the band-limited energy  
119 detector using Raven Pro 1.5 (Cornell Lab of Ornithology; Ithaca, New York) (Charif et al.,  
120 2010). Peak frequency, was measured using the spectrogram slice view at the center of each  
121 estimating chirps (Fig. 2).

122

### Fig. 2

123

124 Removing the background noise was achieved using the Adaptive filtering option.  
125 Spectra were produced using a 512-point Fast Fourier Transform Hamming window in Raven

126 Pro 1.5. A syllable and the minimum interval between adjacent syllables were empirically found  
127 on the sonogram for each recording as a distance between the chirp series exceeding the average  
128 interval between the minimal groups of chirps (three intervals were taken for analysis).

### 129 **Statistical analysis**

130 The listed parameters in each record were measured at 5 points calculated with the  
131 RANDBETWEEN function in Microsoft Excel. If their number did not allow to choose all  
132 possible signals were taken into account. Subsequent comparisons were preceded by averaging the  
133 obtained measurements for each individual. The mean values taken for each recording were  
134 analyzed. Signal parameters were compared using the Kruskal-Wallis test; for statistically  
135 significant differences, multiple comparisons were performed using a Bonferroni–Dunn post hoc  
136 test. All of the statistical analyses were conducted using Statistica 8.0 (StatSoft Inc.; Tulsa,  
137 United States).

138

### 139 **Results**

140 For *P. proximus* and *P. subopacus* males selected for recording, the accuracy of sexual  
141 separation was 100%. Verification of sexual identification of *P. nigrielytris* carried out after  
142 recording of signals and fixation in alcohol was 65% due to less pronounced sexual dimorphism,  
143 in contrast to the other two tested species.

144 We obtained sound recordings of the males stridulation of the three tested species. We  
145 did not try to record any kind of the female song due to absence of stridulatory apparatus on  
146 elytra as it was noted early (Kerchev, 2018) and checked for *P. nigrielytris* on collection  
147 materials of 2015 year. It has been established that *P. nigrielytris* males possess the largest areas  
148 of pars stridens with greatest number of ridges (Table 1).

#### 149 **Table 1**

150 The highest density of ridges in pars stridens was noted for *P. subopacus* (Table 1). Significant  
151 difference was found in the syllable duration and the chirp rate between species ( $H(2, 60) =$   
152  $23.8; p = 0.0000$ ) (Table 2). Nevertheless, the parameter of interchirp intervals duration did not  
153 show the presence of statistically significant differences between the comparing species signal.

#### 154 **Table 2**

155 Significant differences were identified between the signals of the tested species in the  
156 chirp duration parameter ( $H(2, 60) = 15.5; p = 0.0004$ ). In this parameter *P. proximus* and *P.*

157 *nigrielytris* are more distinguishable from *P. subopacus* than between to each other (Table 2).  
158 The value of interchirp intervals in the series is found to be differentiated between species ( $H(2, 60)$   
159  $= 7.2$ ;  $p = 0.03$ ) while no significant differences were observed in the pairwise comparison. The  
160 parameter of the number of tooth strikes/chirp ( $H(2, 60) = 38.8$ ;  $p = 0.0000$ ) showed highest  
161 species specificity. In the pairwise comparison of this signal parameter, statistically significant  
162 differences were found for all the pairs of species compared (Table 2). The interval duration  
163 between tooth strikes did not show significant differences only in the pair of *P. nigrielytris* and  
164 *P. subopacus*.

165

**Fig. 3.**

166

167 Energy is concentrated between 2000 and 22000 Hz, within the human hearing range, with the  
168 two most noticeable peaks about 8 and 14 kHz (Fig. 2). The average values of the main peak of  
169 energy are shown in table 1.

**170 Discussion**

171

172 Insects were collected at the beginning of spring dispersal of the four-eyed fir bark beetle.  
173 Similar to the secondary range (Kerchev, 2014), this species is one of the earliest among the bark  
174 beetles on Sakhalin (Krivolutskaya, 1958). The main part of the beetles of this species was  
175 mature, leaving the birth tree or ready to fly prepupae, pupae, and young beetles with light chitin  
176 were mainly observed in the galleries of *P. subopacus* and *P. nigrielytris* under bark of infected  
177 trees. For recording mature adults were collected from well-lit and heated areas only. Thus, in  
178 addition to host-specificity, phenological isolation can be considered as one of the factors of  
179 interspecific isolation of the test species.

180

181 Behavioral differences between species can be identified through differences in mating  
182 systems. *P. subopacus* is the only harem-poligynous among the three species compared. Sex  
183 ratio in its families is about 2–5 females per male (Stark, 1952). The families of the other two  
184 species are monogynous. Sexual behavior of the *P. proximus* was previously discussed (Kerchev,  
185 2014), whereas data on the characteristics of sexual behavior of *P. nigrielytris* are given for the  
186 first time. During insect collection, only a pair of parent beetles was always found in nests  
187 inhabited by beetles in spite of the fact that the number of egg galleries was 1–4.

187

188 The morphological characteristics of the stridulatory apparatus of the *P. proximus* were  
189 reported earlier (Sasakawa & Yoshiyasu, 1983), after which they were specified and



189 supplemented (Kerchev 2015). The presence of the stridulatory apparatus in *P. subopacus* was  
190 identified for the first time more than a hundred years ago, but the morphology description was  
191 not provided (Witchman 1912; Lyal and King, 1996). For *P. nigrielytris*, this study indicates the  
192 presence of stridulation and the morphological features of the structures involved in the sound  
193 production for the first time (Table 1). In general, the species of the genus *Polygraphus* Er. are  
194 similar in the morphology of the stridulatory apparatus, but different in morphometric features.  
195 Intraspecific comparison shows variations in the area and the number of ridges in pars stridens  
196 (Kerchev, 2015), and the density of ridges per unit length of the area can be noted as a more  
197 stable feature.

198 Like many other insects, bark beetles are physically limited in the production of sounds  
199 due to their small size (Bennet-Clark, 1998). The studied species exhibit noticeable differences  
200 in the relative amplitude of signals, which is most likely due to the insect size (Table 1),  
201 especially in *P. subopacus* with other two bigger species (Fig. 3). Among cicadas and crickets,  
202 the smallest species produce signals with highest frequency compared to those by larger species.  
203 A similar negative correlation between body size and frequency parameters was noted earlier for  
204 bark beetles of the genus *Dendroctonus* Er. (Yturralde and Hofstetter. 2015). The study revealed  
205 significant differences between stridulatory signals of the studied species in five of the seven  
206 temporal parameters. No differences were found in parameters such as number of chirps per  
207 syllable and duration of intervals between them.

208 Signal parameters showed the highest specificity, starting with the level of chirp, which is  
209 primarily due to the physiological characteristics of the species and the morphology of the  
210 microstructures of their stridulatory apparatus (Yturralde and Hofstetter. 2015; Kerchev, 2018).

211 It was experimentally found that a rather short fragment of the signal consisting of 14  
212 pulses repeated at least once a minute is sufficient for females of the bush-cricket *Metrioptera*  
213 *roeselii* Hagen. to recognize an intraspecific attractive signal (Zhantiev and Korsunovskaya,  
214 2014). In the case of competitive interaction between *P. proximus* males, the contact lasts more  
215 than a minute only during the fight for a female boring into the bark. In other cases, the male  
216 stays at the entrance to the gallery occupied by a formed family for not more than several  
217 seconds (Kerchev, 2018). Consequently, the territorial signal must have the characteristics that  
218 would allow it to be recognized in a short period, and the chirp as a signal unit has all the

219 necessary characteristics. Under experimental conditions, continuous stridulation may be caused  
220 by limited abilities to escape contact between individuals inside the arena.

221 As already a number of different ethological supports mentioned, interspecific isolation  
222 and communication features. Species-specificity of stridulatory signals may be an additional  
223 parameter that performs the same role for individuals that have started to populate a tree beyond  
224 the main dispersal flight period. In interaction of males of different species, the signal receiver  
225 may not regard it as a repellent. A clear repellent reaction during conspecific interaction can  
226 indicate a crucial role of these signals in reduction of intraspecific competition.

227 To date, a number of research papers (Mankin et al., 2008; Potamitis et al., 2008;  
228 Schofield & Chesmore 2010) are devoted to the use of species-specific insect signals for species  
229 identification. The possibility of identification of alien species and monitoring of their  
230 populations based on detection of their species-specific signals is of particular interest in this  
231 regard. Among the parameters tested, the most relevant parameters are syllable duration, the  
232 interval between syllables, the number of syllables per unit of time, and the relative amplitude of  
233 signals.

234

## 235 **Conclusions**

236 The study showed that temporal parameters of the intraspecific signals of the test species  
237 exhibit significant differences in a number of characteristics, not only between sympatric species,  
238 but also with the species that has a clear distinction in the host plant.

239 The species-specificity of stridulatory signals may be an additional parameter for  
240 reproductive isolation of species that occasionally occur on the same tree species. Reception and  
241 reaction to this type of signals may be present both at the interspecific level of interactions and  
242 during intraspecific contacts only. To verify the possibility of interspecific communication at the  
243 level of one genus, it is necessary to conduct playback experiments with recording of responses  
244 to alternating con- and interspecific signals. Biologically, signals produced by males of one  
245 species may reduce intraspecific competition at high population density

246 Of particular interest is the possibility of using species-specific characteristics of acoustic  
247 signals of bark beetles for identification and detection of alien species. Among the tested  
248 parameters of stridulation signals, the following specific characteristics can be distinguished for  
249 the genus *Polygraphus*: chirp duration, number of tooth-strikes per chirp, and intertooth-strike

250 interval. This method can be used universally one to compile libraries of species-specific signals  
251 in order to further develop methods for detection and species identification of bark and wood-  
252 boring pests.

253

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258

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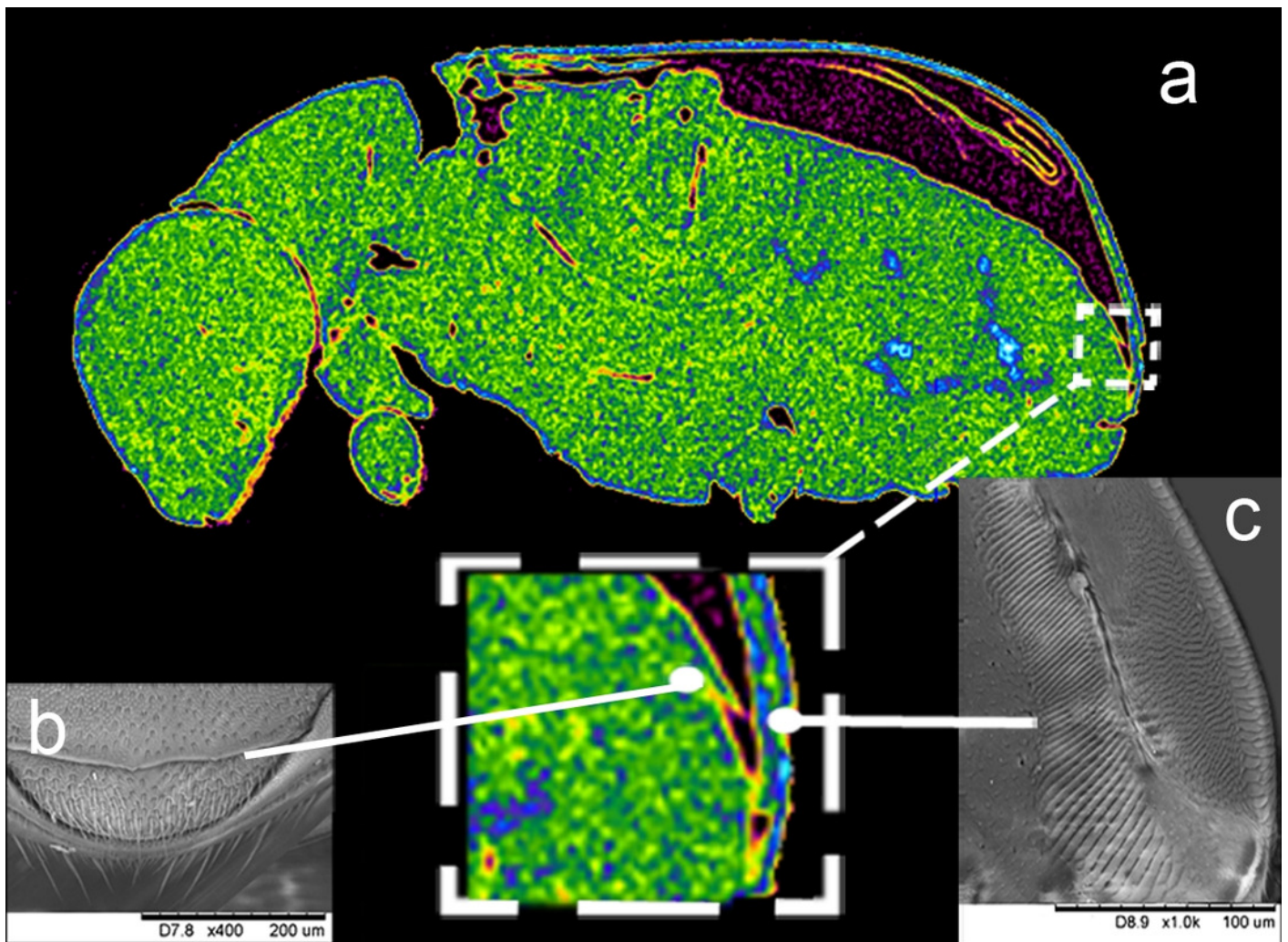
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# Figure 1

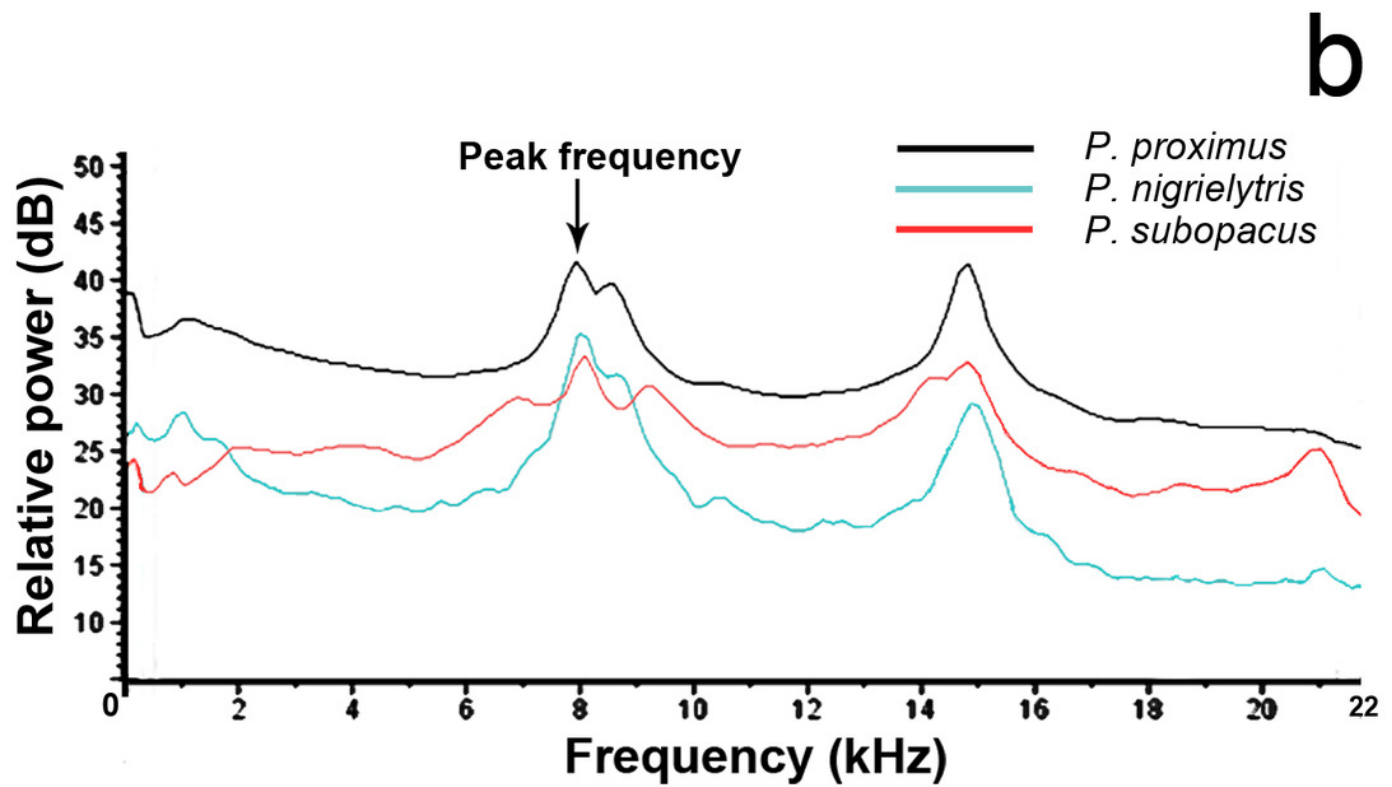
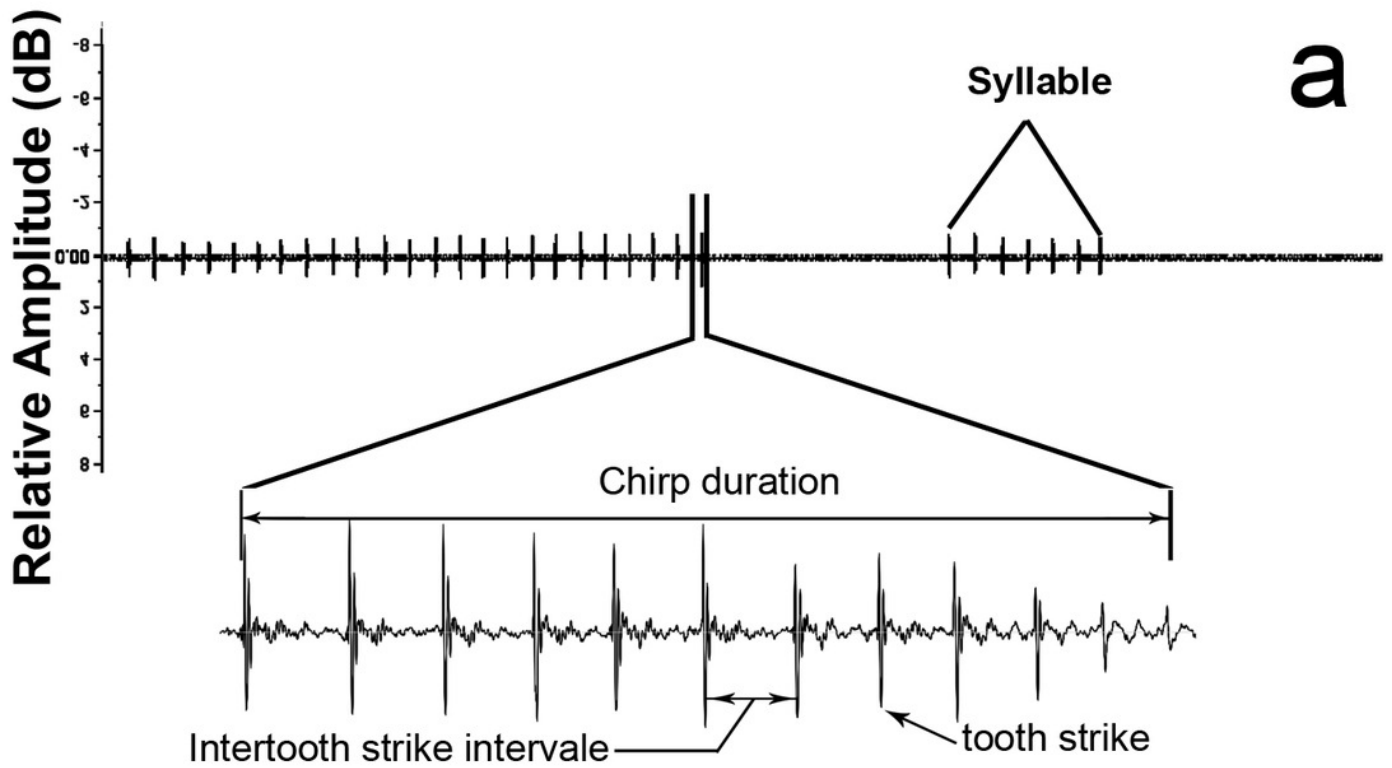
Fig 1. a - longitudinal section of the imago prepared using an X-ray microtomography device; image of the elytron-tergite stridulatory apparatus of males; b - detailed structure of the plectrum; c - pars stridens structure.





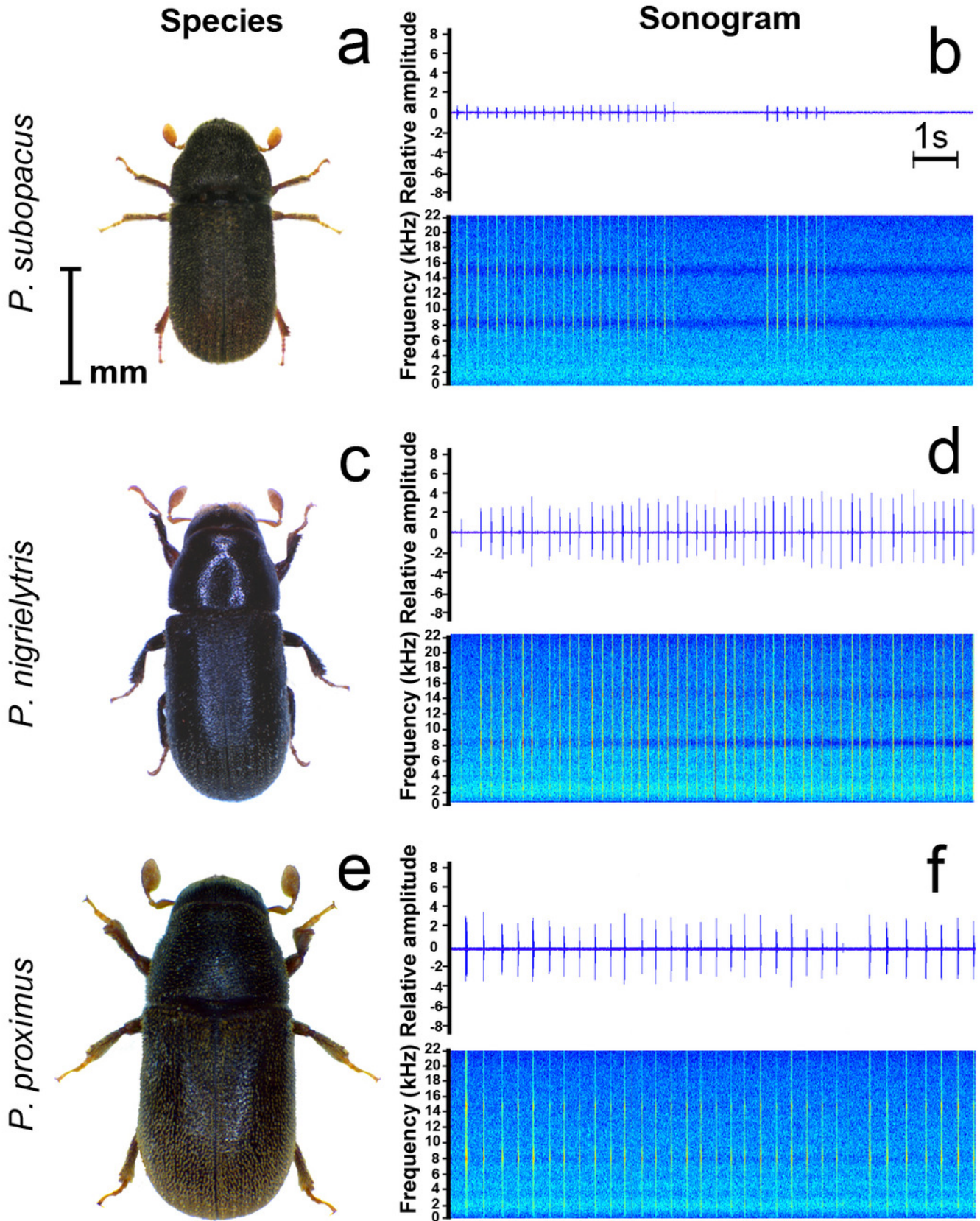
## Figure 2

Descriptions of temporal and frequency-amplitude parameters measured in male stridulatory signals of *Polygraphus proximus*, *P. nigrielytris* and *P. subopacus* (a - descriptions of temporal parameters; b - frequency-amplitude parameters of ma



## Figure 3

Adults of tested species with fragments of their signal samples (a - imago of *Polygraphus proximus*, c - *P. nigrielytris*, e - *P. subopacus*; b, d, f - their sonograms respectively)



**Table 1** (on next page)

Morphological characteristics of the stridulatory apparatus and average values of the signal parameters in males of *Polygraphus proximus*, *P. nigrielytris* and *P. subopacus* (Mean  $\pm$  SD).

1 Table 1. Morphological characteristics of the stridulatory apparatus and average values of the  
 2 signal parameters in males of *Polygraphus proximus*, *P. nigrielytris* and *P. subopacus* (Mean  $\pm$   
 3 SD).

4

| Parameter  | <i>P. proximus</i> | <i>P. nigrielytris</i> | <i>P. subopacus</i> |
|--|--------------------|------------------------|---------------------|
| Stridulating sex                                 | male               | male                   | male                |
| Length of beetle (mm)                            | 2.5 $\pm$ 0.2      | 2.7 $\pm$ 0.1          | 1.9 $\pm$ 0.1       |
| Length of pars stridens left/right ( $\mu$ m)    | 156/193            | 198/ 213               | 148/ 152            |
| Number of rows in pars stridens                  | 55/53              | 71/76                  | 65/66               |
| Syllable duration (s)                            | 14.4 $\pm$ 8.4     | 4.4 $\pm$ 2.7          | 8.4 $\pm$ 6.1       |
| Number of chirps /syllable                       | 14.8 $\pm$ 18.1    | 19.1 $\pm$ 13.3        | 26.3 $\pm$ 21.4     |
| Chirps rate (chirps/s)                           | 5.1 $\pm$ 0.9      | 4.4 $\pm$ 0.9          | 3.9 $\pm$ 0.9       |
| Chirps duration (s)                              | 0.025 $\pm$ 0.008  | 0.020 $\pm$ 0.007      | 0.042 $\pm$ 0.001   |
| Interchirp interval (s)                          | 0.17 $\pm$ 0.4     | 0.23 $\pm$ 0.04        | 0.26 $\pm$ 0.05     |
| Number of tooth-strikes                          | 13.4 $\pm$ 4.0     | 10.5 $\pm$ 3.2         | 7.5 $\pm$ 2.2       |
| Intertooth-strikes interval (s)                  | 0.002 $\pm$ 0.0006 | 0.001 $\pm$ 0.0005     | 0.004 $\pm$ 0.001   |
| Peak frequency (kHz)                             | 7960.7 $\pm$ 42.27 | 8017,95 $\pm$ 65,21    | 8715 $\pm$ 2113     |
| Relative power of signal (dB) at 1.5 cm distance | 41.7 $\pm$ 5.3     | 34.2 $\pm$ 7.0         | 33.0 $\pm$ 5.3      |

5

**Table 2** (on next page)

Results of pairwise comparison of parameters of competitive signals in males of *Polygraphus proximus*, *P. nigrielytris* and *P. subopacus*.

1 Table 2 Results of pairwise comparison of parameters of competitive signals in males of  
 2 *Polygraphus proximus*, *P. nigrielytris* and *P. subopacus*.

3

| Parameter                      | Species             | <i>P. subopacus</i> | <i>P. nigrielytris</i> |
|--------------------------------|---------------------|---------------------|------------------------|
| Syllable duration (s)          | <i>P. proximus</i>  | 2.6                 | 4.9**                  |
|                                | <i>P. subopacus</i> |                     | 2.3                    |
| Chirp rate (chirp/s)           | <i>P. proximus</i>  | 3.2*                | 1.3                    |
|                                | <i>P. subopacus</i> |                     | 2.1                    |
| Chirp duration (s)             | <i>P. proximus</i>  | 3.6**               | 3.1*                   |
|                                | <i>P. subopacus</i> |                     | 3.6**                  |
| Tooth strikes /chirp           | <i>P. proximus</i>  | 6.2**               | 3.1*                   |
|                                | <i>P. subopacus</i> |                     | 3.1*                   |
| Intertooth strike interval (s) | <i>P. proximus</i>  | 3.2*                | 0.3                    |
|                                | <i>P. subopacus</i> |                     | 3.4**                  |
| Peak frequency (kHz)           | <i>P. proximus</i>  | 3.4**               | 2.7                    |
|                                | <i>P. subopacus</i> |                     | 0.7                    |
| Relative power of signal       | <i>P. proximus</i>  | 4.2**               | 3.3**                  |
|                                | <i>P. subopacus</i> |                     | 1.0                    |

4 Z-values in cells, \*p<0.05; \*\*p<0.01 with Bonferroni correction

5