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1 Highly diversified circadian rhythms in the calling activity of eneopterine crickets
2 (Orthoptera: Grylloidea: Gryllidae) from Southeast Asia

3

4 Ming Kai Tan^{1*} and Tony Robillard¹

5

6 ¹ Institut de Systématique, Evolution et Biodiversité (ISYEB), Muséum national d'Histoire
7 naturelle, CNRS, SU, EPHE, UA, 57 rue Cuvier, CP 50, 75231 Paris Cedex 05, France

8 *Corresponding author: orthoptera.mingkai@gmail.com

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10

11

12 **Abstract**

13 Circadian rhythm is an endogenously-generated and self-sustaining oscillations that occur at
14 a 24 hours scale and is ubiquitous among animals. Animals rely on their circadian clocks to
15 anticipate cyclical external environmental oscillations to find mates and reproduce, among
16 other activities. Hence, animals that sing to attract mates can modulate their calling activity
17 across the circadian cycle and species can exhibit different circadian rhythms in their calling
18 activity. Crickets are among the most studied sound-producing animals but concerted effort
19 to examine their circadian rhythms in their calling activity across multiple species remains
20 limited for Southeast Asian species. Here, we recorded the calls of eleven Southeast Asian
21 species of Eneopterinae—known for their diversified calling songs—across the phylogeny
22 and compared their circadian rhythms based on the number of echemes and amount of sound
23 produced. Using standardised ex-situ recordings to produce precise data about the circadian
24 patterns, we verified sporadic observations about their calling activity made in the field. We
25 also found that the eneopterines exhibit highly diversified circadian rhythms in the calling
26 activity, ranging from strictly diurnal and nocturnal calling to complex calling patterns with
27 multiple peaks across day and/or night.

28

29 **Key words:** acoustics, calling song, Eneopterinae, high frequency, temporal

30

31

32 **Introduction**

33

34 Circadian rhythm refers to endogenously-generated and self-sustaining oscillations occurring
35 on an approximately 24-hours scale (Dunlap, 1999; Bell-Pedersen et al., 2005). Such rhythms

36 originate within cells that serve as taxon-specific ‘pace-makers’ or ‘molecular clocks’ and
37 can drive cyclical responses in biochemistry, physiology and behaviour (Gwinner &
38 Brandstatter, 2001). Circadian rhythms are ubiquitous among animals and circadian clocks
39 have been demonstrated to be critical for adaptive functions (Helfrich-förster et al., 1998;
40 Yerushalmi & Green, 2009). Animals rely on their circadian clocks to anticipate the daily
41 external cyclical environmental oscillations and prepare themselves—both physiologically
42 and behaviourally—to find food and mates, to reproduce and take care of their young, and to
43 migrate (Marques & Waterhouse, 1994; Helfrich-förster et al., 1998; Yerushalmi & Green,
44 2009).

45

46 For species that produce sound to attract mates, species-specific cyclic variations in the
47 calling activity across the circadian cycle is likely to be governed by an intrinsic circadian
48 clock that enables synchronous mate-finding by males and females (Helfrich-förster et al.,
49 1998). This synchrony is essential for sound-producing animals to balance reproduction and
50 survival: maximising chances of attracting mates while minimising predation risk (Fenn &
51 MacDonald, 1995). Nonetheless, calling activity can also be plastic owing to the influence of
52 environmental factors (e.g., synchronisers or zeitgebers) and presence of potential mates or
53 male competitors (e.g., masking agents) (Helfrich-förster et al., 1998; Yerushalmi & Green,
54 2009). Under consistent risk–reward conditions, animals can evolve species-specific
55 behaviours to fit particular ecological niches, e.g., diurnal *vs.* nocturnal singing (Honegger
56 1981; Fergus & Shaw 2013; Symes et al., 2020).

57

58 Circadian patterns can lead to many different biological outcomes. For example, circadian
59 patterns may allow organisms to escape predators, parasites, or acoustic competitors (Taylor

60 & Friesen, 2017). Divergence in the circadian rhythms can also lead to shifts in diel breeding
61 times, initiating and/or driving allochronic separation and potentially speciation (Taylor &
62 Friesen, 2017). Examination of temporal variation in calling activity of a broad range of
63 understudied animals can therefore provide insights into their basic ecology and evolution of
64 calling behaviours (Clink et al., 2021). In the current era when the natural world is heavily
65 perturbed by human activities, studying the circadian rhythms is also important to understand
66 how human disturbance (e.g., light pollution) can disrupt circadian rhythms in animals and
67 the consequences of such disruptions on their behaviours, ecology and survival (Kumar et al.,
68 2017; Jiang et al., 2020).

69

70 Being one of the most readily studied species with bioacoustics, crickets and other
71 orthopteroid insects have been subjected to research on the endogenous mechanisms
72 controlling the circadian rhythms, ranging from physiological to molecular investigations
73 (e.g., Tomoika & Chiba, 1982; Helfrich-förster et al., 1998; Fergus & Shaw 2013; Uryu et al.,
74 2013). While there are plenty of studies looking into circatidal activity rhythm in the coastal
75 species, *Marinemobius asahinai* (Yamasaki, 1979) (e.g., Satoh et al., 2008; Takekata et al.,
76 2014; Sakura et al., 2017), these crickets are mute. The circadian rhythms of the calling
77 activity in crickets have been described and studied intensively in a few model species, such
78 as *Teleogryllus* and *Gryllus* ssp. (e.g., Werrer et al., 1985; Loher, 1972; French & Cade, 1987;
79 Rost & Honegger, 1987; Wade et al., 1987; Wiedenmann et al., 1988) and sporadically in
80 other orthopterans (see e.g., Walker & Whitesell, 1982; Heller & von Helversen, 1993;
81 Danley et al., 2007; Diwaker & Balakrishnan, 2007; Franklin et al., 2009; Singh & Jain, 2021;
82 Symes et al., 2020). While some of these studies were field-based (Rost & Honegger, 1987;
83 Greenfield, 1988; Singh & Jain, 2021), other incorporated laboratory experiments (Loher &

84 Orsak, 1985; Werrer et al., 1985). Some of these studies focused on single species (Walker &
85 Whitesell, 1982; Rost & Honegger, 1987; Singh & Jain, 2021) and others on communities
86 (French & Cade, 1987; Danley et al., 2007; Diwaker & Balakrishnan, 2007; Symes et al.,
87 2020).

88

89 Most of the previous studies focused on species from the temperate regions (e.g., French &
90 Cade, 1987), the Neotropics (e.g., Symes et al., 2020) and other biodiverse regions (e.g.,
91 Danley et al., 2007; Diwaker & Balakrishnan, 2007) outside of Southeast Asia. But for the
92 highly speciose cricket clades from Southeast Asia, there is however still a dearth of
93 information about their circadian activity. Despite some anecdotal observations on the
94 activity level of crickets—especially *in-situ* ones, they may not give a complete
95 understanding. There has also not been a comparative study on the circadian rhythms of
96 calling activity across the phylogeny of orthopterans from Southeast Asia (see French &
97 Cade, 1987).

98

99 The acoustic communication of the Eneopterinae subfamily of crickets has been well-studied
100 (e.g., Robillard & Desutter-Grandcolas, 2004a, 2004b, 2011a, 2011b; Robillard et al., 2013,
101 2015; ter Hofstede et al., 2015; Benavides et al. 2020). This subfamily is also highly
102 diversified in Southeast Asia. The calling songs these eneopterines have been recorded and
103 described, unlike many other subfamilies of crickets from this region (e.g., Robillard & Tan,
104 2013; Robillard et al., 2014; Tan et al., 2019). From these sporadic observations, some
105 eneopterines could be broadly classified as diurnal (e.g., *Nisitrus* and *Eneoptera*) and others
106 as nocturnal (e.g., *Cardiodactylus* and *Agnotecous*) (e.g., Desutter-Grandcolas et al., 2010;
107 Robillard & Tan, 2013; Robillard et al., 2014; Tan & Robillard, 2014; Tan et al., 2019).

108 These data can be refined and extended by generating more precise data on the entirety of the
109 circadian patterns, resulting in much more nuanced classification than a simple designation of
110 diurnal or nocturnal).

111

112 To address these knowledge gaps, we recorded the calling activity in a range of crickets in
113 isolation, in a controlled *ex-situ* environment that removed the potential confounding
114 zeitgebers and masking factors (i.e., environmental noises, female presence, callings by other
115 males or other species). We focused particularly, species from the tribe Nisitrini and
116 Lebinthini because these clades are most speciose in the region. Given that the call
117 parameters are highly diversified (Robillard & Desutter-Grandcolas, 2011), we assessed how
118 variable circadian rhythms can be among different eneopterine species. We postulated that
119 the circadian rhythms can also be diverse including variations in the active periods (e.g.,
120 narrow *vs.* broad active period) across different species. Based on previously published
121 natural history observations in the field, we expected the 24-hours recordings under
122 controlled environment to unveil that while some species are strictly diurnal or nocturnal,
123 other species call across the 24 hours.

124

125

126 **Materials and Methods**

127

128 *Lab colonies*

129 Breeding colonies in the lab were established with cricket eggs collected during fieldwork
130 between 2011 and 2019. Lab colonies of eneopterines were housed and maintained in large
131 tanks at a temperature-controlled (25–27°C) insect rearing room of the Muséum national

132 d'Histoire naturelle, Paris (MNHN). The crickets in the insect rearing room were subjected to
133 11:13 bright: dark hours reflecting day duration in the collection sites. Adult males and
134 females, as well as juveniles of the same species were kept together in small communities as
135 they were observed in their natural environment. These community tanks vary in size (60 cm
136 length × 50 cm height × 40 cm width, 60 cm length × 40 cm height × 40 cm width or 40 cm
137 length × 25 cm height × 20 cm width), depending on the size of the species and the number
138 of individuals in the tanks. We ensured that there was no overcrowding so that there were
139 sufficient food and space for the inhabitants of these community tanks at all time. Moist soil,
140 European ivy (*Hedera helix*) and large water-soaked cotton balls were provided to prevent
141 dehydration and maintain the high humidity the crickets are exposed to in their natural
142 environment. The crickets were fed *ad libitum* with European ivy, Affinity ULTIMA Mini
143 Adult dog pellets food and BJORG Muesli whole grain cereals without added sugar.
144
145 Male adults were opportunistically collected from these community tanks for the recording.
146 For each species, recordings for individuals from the same generation were done within the
147 same period of time over a few weeks to minimise confounding effect of generation and age
148 of the crickets.
149
150 To record the calling adult cricket under standardised conditions, we placed an isolated male
151 in an insect cage with nylon netting (25 cm in diameter and 33 cm tall) inside a temperature-
152 controlled sound attenuating room in MNHN (2.2 m height, 2.2m width, 2.9 m length) and
153 recorded its acoustic activity for 24 hours each with 11:13 bright: dark hours similar to that in
154 the insect rearing room. Temperature was maintained between 26 to 28°C using an Eqiva
155 Universal-Thermostat (model: UT300, eQ-3, Leer, Germany). We used a HOBO 8K

156 Pendant® Temperature logger (model: UA-001-08, Onset, Bourne, MA) to track the room
157 ambient temperature once every half an hour. Humidity within the cage was maintained with
158 cotton ball soaked in water. We used a condenser capsule microphone CM16 (Avisoft
159 Bioacoustics, Berlin, with a flat frequency response from 3 to 150 kHz) placed directly beside
160 the cage for recording. Automatic recordings were made using the program Avisoft
161 Triggering Harddisk Recorder version 2.97 and an 8-Pre MOTU sound card at a sampling
162 frequency of 96 kilo-samples per second (16 bit). Recording was automatically triggered
163 when sound was detected at 3.8% of full scale. All sound 5 s prior to the trigger was recorded
164 and saved, and the recording continued for 30 s after the last sound had been detected. These
165 recordings containing the complete calling songs were saved as 16-bit WAV sound files. The
166 start time of each sound file was recorded as part of the file name, so that we were able to
167 determine the time at which each calling song was produced. Each male was recorded once
168 over 24 hours. In total, we recorded 128 males from nine species and six genera across the
169 eneopterine phylogeny (Table 1). The highly variability in the numbers of males recorded for
170 each species was attributed to different species having different brood size, colony size and
171 survival rate in captivity.

172

173 ***Wild individuals***

174 Wild individuals were also recorded to supplement the data collected from colonies. Active
175 male individuals without damaged wings were opportunistically collected from different parts
176 of Singapore where *Cardiodactylus admirabilis* and *Lebinthus luae* are found (Robillard &
177 Tan, 2013; Tan & Robillard, 2014): Admiralty Park (for *C. admirabilis*); Hindhede Nature
178 Park, Labrador Nature Reserve and Pulau [= island in Bahasa Malayu] Ubin (all for *L. luae*).
179 In total, we recorded 35 males from the two species (Table 1).

180

181 To record the calling adult crickets under standardised laboratory conditions, we placed an
182 isolated male in an insect cage with nylon netting (25 cm in diameter and 33 cm tall, similar
183 in size as the one used in MNHN) inside a sound attenuating box (dimension 55 cm length ×
184 40 cm width × 45 cm height). For each male, we recorded its acoustic activity continuously
185 for 24 hours with 11:13 bright: dark hours using a Wildlife Acoustics Song Meter SM4 with
186 built-in microphones and the following settings: sampling frequency of 96 kilo-samples per
187 second (16 bit), no compression, filter at 1 kHz, and preamp 26 dB, gain 5.0 dB. Each 16-bit
188 WAV sound file was 1 hour long with the start time of each sound file recorded as part of the
189 file name. We used a HOBO 8K Pendant® Temperature logger (model: UA-001-08, Onset,
190 Bourne, MA) to track the ambient room temperature every 30 minutes. Humidity within the
191 cage was maintained with a cotton ball soaked in water.

192

193 *Acoustic and statistical analyses*

194 From the sound files, we determined the time at which each echeme was recorded. We then
195 counted the total number of echemes per hour. An echeme typically consists of a group of
196 syllables and corresponds to a call unit in terms of communication. A syllable corresponds to
197 the sound produced by one closing movement of the cricket forewings (Ragge & Reynolds,
198 1998; Baker & Chesmore, 2020). The greater the number of echemes per hour, the higher the
199 calling activity per hour. For *Nisitrus* species that produce long sequences of very short
200 echemes (each consisting of a triplet of syllables) repeating in close tandem (Fig. 1(a)), we
201 quantified echeme sequences (sensu Baker & Chesmore, 2020) because it corresponds more
202 biologically functional as the call unit for communication. It is more likely that females
203 recognise the entire echeme sequence as a call unit rather than each single echeme.

204

205 Additionally, counting the number of echemes produced per hour may not be sufficient to
206 understand the level of calling activity of the cricket per hour. This is because different
207 species produce echemes with different echeme duration and number of syllables, as well as
208 with high or low duty cycles. Hence, we also estimated the duration of sound produced per
209 hour by multiplying the number of echemes per hour with the average duration of sound
210 produced in a single echeme for the species (following the approach by Symes et al., 2020).
211 The average amount of sound produced in a single echeme, which is distinct from sound
212 pressure level and duty cycle, was quantified for each species by summing the durations of
213 the individual syllables within each echeme (and excluding the down times within each
214 echeme) (Symes et al., 2020). These were done manually by visualising the sound files using
215 Raven Lite 2.0.0.

216

217 All statistical analyses were conducted with R version 3.5.1 (R Development Core Team,
218 2018). To obtain smooth lines to visualise the circadian rhythm of the call activity for each
219 species, we first fitted generalised additive mixed effects models (GAMMs) with
220 quasipoisson error structure for the number of echemes per hour using the function ‘`gamm`’
221 from the R package ‘`mgecv`’ (Wood & Wood, 2015). Quasipoisson error structure was chosen
222 to account for overdispersion of count data. The cricket ID was fitted as a random intercept
223 (to control for individual differences) and hour (time of the day) as a smoothed fixed effect.
224 Cyclic cubic smoothing spline (`bs = “cc”`) was used as the smoothing method to estimate
225 periodic variation. We then fitted GAMMs with gaussian error structure for the amount of
226 sound produced per hour with the same smoothed fixed and random effects. Based on the
227 smooth lines, we describe the circadian rhythms by identifying the peaks and minimums in

228 call activity as well as the corresponding number of echemes and amount of sound produced
229 (\pm standard deviation). We also determined the peak width at 50% of its maximum call
230 activity.

231

232

233 **Results**

234

235 In total, we recorded 137 males from 11 species of eneopterines based on both laboratory
236 colonies and wild individuals (Table 1). The two *Nisitrus* species have similar calling songs
237 consisting of echeme sequences made up of a series of echemes comprised of triplets of
238 syllables (Figure 1(a)). The echeme of *Cardiodactylus palawan* consists of three to 17
239 consistently-spaced syllables; of *C. admirabilis* consists of three to four syllables (Figure
240 1(b)). *Gnominthus baitabagus* (Figure 1(c)), *Macrobinthus jharnae* (Figure 1(d)),
241 *Microbinthus pintaui* (Figure 1(e)), *Lebinthus sanchezi* (Figure 1(f)) and *Lebinthus*
242 *sandakan* (Figure 1(g)) have a similar calling song structure, i.e., each echeme consisting of a
243 series of closely-spaced syllables (mean number of syllables—*G. baitabagus* = 57 ± 13
244 [number of echemes = 86]; *M. jharnae* = 180 ± 59 [n = 524]; *M. pintaui* = 295 ± 68 [n = 690];
245 *L. sanchezi* = 52 ± 8 [n = 313]; *L. sandakan* = 344 ± 397 [n = 358]). *Lebinthus bitaeniatus* and *L.*
246 *luae* have calling songs distinct from the nine other species: each echeme consists of initial
247 well-spaced syllables followed by a short trill (Figure 1(h)).

248

249 *Diurnal species*

250 The activity patterns for *N. malaya* and *N. vittatus* indicate that they are diurnal species,
251 although the former occasionally sing before dawn and after dusk (Figures 2(a), 2(b), 3(a),

252 3(b)). Both species produce low number of echemes per hour comparatively to other species,
253 but the amount of sound produced per hour was higher than most species. On average, *N.*
254 *malaya* calls from the 5:00 to 20:00 hours (15 hours), slowly peaking to ca. 17 ± 1 echeme
255 sequences per hour at the 16:00 hour (50% of peak [9:00, 15:00]) (Figure 2(a)), but the
256 amount of sound produced per hour peaks steeply to ca. 1271 ± 9 s at the 15:00 hour (50% of
257 peak [9:00, 19:00]) (Figure 3(a)). On average, *N. vittatus* calls fairly consistently throughout
258 from the 6:00 to 19:00 hours (13 hours), peaking at the 16:00 hour (50% of peak [9:00,
259 18:00]) with ca. 9 ± 1 echeme sequences per hour (Figure 2(b)), but the amount of sound
260 produced per hour peaks steeply to ca. 1376 ± 21 s at the 15:00 hour (50% of peak [10:00,
261 18:00]) (Figure 3(b)).

262

263 Within Lebinthini, *L. sandakan* is another diurnal species (Figures 2(c), 3(c)). On average,
264 this species calls in a relatively narrow time period, from 8:00 to 14:00 (6 hours). Calling
265 activity increases sharply to a distinct peak of ca. 37 ± 4 echemes and ca. 388 ± 26 s per hour at
266 the 11:00 hour (50% of peak [10:00, 12:00]), before decreasing sharply (Figures 2(c), 3(c)).

267

268 *Nocturnal species*

269 On the other hand, *M. jharnae* is a strictly nocturnal species (Figures 2(d), 3(d)). On average,
270 this species calls throughout night-time from the 17:00 to 6:00 hours (13 hours). Based on the
271 smoothed trend line, calling activity peaks at ca. 21 ± 1 echemes per hour at the 1:00 hour (50%
272 of peak [21:00, 3:00]) (Figure 2(d)) while the amount of sound produced per hour peaks at ca.
273 77.3 ± 0.7 s per hour at the 0:00 hour (50% of peak [20:00, 4:00]) (Figure 3(d)).

274

275 *Cardiodactylus admirabilis* is another strictly nocturnal species. On average, this species
276 calls throughout night from the 18:00 to 4:00 hours (10 hours) (Figure 2(e), 3(e)). Calling
277 activity peaks between the 21:00 and 0:00 hours (Figures 2(e), 3(e)). At the peak of its
278 activity, this species produces a high number of echemes per hour but low amount of sound
279 produced per hour relative to other species.

280

281 *Species with complex patterns*

282 From the smooth line based on the number of echemes, *C. palawan* calls at relatively narrow
283 time periods, once during daytime and once at dusk (Figure 2(f)). On average, the calling
284 activity increases sharply to a distinct peak at the 12:00 hour (50% of peak [11:00, 14:00])
285 before decreasing sharply to a minimum at the 15:00 hour (Figure 2(f)). Call activity then
286 increases sharply again to a second distinct peak at the 19:00 hour (50% of peak [17:00,
287 21:00]), followed by a sharp decrease in calling activity (Figure 2(f)). However, from the
288 smooth line based on the amount of sound produced, *C. palawan* calls across the day and
289 night, starting after dawn and increasing gradually to a maximum at the 18:00 and 19:00
290 hours (50% of peak [10:00, 23:00]) (Figure 3(f)). Similar to *C. admirabilis*, *C. palawan*
291 produces a high number of echemes per hour but low amount of sound produced per hour
292 relative to other species.

293

294 *Gnominthus baitabagus* calls in a relatively narrow time period, once during daytime and
295 once during nighttime, from the smooth lines based on both the number of echemes and
296 amount of sound produced (Figures 2(g), 3(g)). On average, the calling activity increases
297 sharply to a distinct peak at the 15:00 hour (50% of peak [13:00, 18:00]) before decreasing
298 sharply to a minimum at the 20:00 hour (Figures 2(g), 3(g)). Calling activity then increases

299 sharply again to a second distinct peak at midnight (50% of peak [22:00, 2:00]), followed by
300 a sharp decrease in calling activity (Figures 2(g), 3(g)). Calling activity ceases between the
301 5:00 and 6:00 hours (Figures 2(g), 3(g)). During the peaks, *G. baitabagus* produces the
302 highest number of echemes and amount of sound produced among the recorded Lebinthini
303 species.

304

305 The circadian rhythms in the calling activity of *L. bitaeniatus*, *L. luae* and *L. sanchezi* are
306 more complex. These species calls throughout the day and night but with undulating activity
307 levels. On average, the calling activity of *L. bitaeniatus* peaks at the 14:00 hour with ca. 36 ± 1
308 echemes and ca. 90.1 ± 0.7 s per hour, then drops steeply to a minimum of ca. 4 ± 0 echemes
309 and ca. 6.2 ± 0.7 s per hour at the 19:00 hour (Figures 2(h), 3(h)). Nocturnal calling activity
310 then increases slightly to a peak of ca. 13 ± 0 echemes and ca. 33.9 ± 0.3 s per hour at the 23:00
311 hour (Figures 2(h), 3(h)). Calling activity subsequently increases to ca. 28 ± 0 echemes and ca.
312 74.1 ± 1.5 s per hour by the 7:00 hour and remains relatively constant before reaching the
313 diurnal peak at the 14:00 hour (Figures 2(h), 3(h)).

314

315 The circadian rhythms in the calling activity of *L. luae* are very similar to that of the sister
316 species *L. bitaeniatus*, with three peaks in calling activity: ca. 60 ± 41 echemes and ca.
317 36.7 ± 3.7 s per hour around dawn (7:00 hour), ca. 46 ± 46 echemes and ca. 25.7 ± 0.5 s per hour
318 in the afternoon (14:00 hour) and ca. 24 ± 32 echemes and ca. 20.1 ± 1.3 s per hour at night
319 (2:00 hour) (Figures 2(i), 3(i)).

320

321 On average, the calling activity of *L. sanchezi* peaks once at the 9:00 hour with ca. 35 ± 0
322 echemes per hour, then drops gradually to ca. 24 ± 0 echemes and ca. 30.3 ± 0.5 s per hour at

323 the 15:00 and 16:00 hours (Figures 2(j), 3(j)). Subsequently, calling activity increases
324 gradually till the 22:00 hour with ca. 48 ± 0 echemes and ca. 58.9 ± 0.3 s per hour (Figures 2(j),
325 3(j)).

326

327 *Species without clear patterns*

328 Unlike other eneopterines, *M. pintaudi* does not show any distinct peaks or troughs in their
329 circadian rhythm in the calling activity (Figure 2(k)). On average, this species calls for ca.
330 7 ± 1 bouts per hour. There were also considerable individual differences: while most
331 individuals indeed call consistently across 24-hours, there are a few individuals that show a
332 peak at slightly after the 10:00 hour and a single individual showing a distinct anomaly in its
333 circadian rhythm.

334

335

336 **Discussion**

337 *Comparative and field observations*

338 Here, we have measured and described for the first time the circadian rhythms of the calling
339 activity in 11 species of eneopterine crickets from Malay Peninsula, Borneo, the Philippines
340 and New Guinea (Table 2). The studied species belong to two tribes across the phylogeny of
341 Eneopterinae and show vastly different circadian rhythms, indicating that different species
342 employ different strategies in modulating their calling activity (Fig. 4). While sister species
343 (i.e., *N. vittatus* and *N. malaya*; *L. bitaeniatus* and *L. luae*) tend to have similar circadian
344 rhythms, diversified circadian rhythms can be observed within speciose genera (e.g.,
345 *Cardiodactylus* and *Lebinthus*) or closely-related genera (e.g., *Macrobinthus* and
346 *Microbinthus*) (Fig. 4).

347

348 We knew from previous field observations that the *Nisitrus* species sing on plant foliage from
349 early morning to dusk (Robillard & Tan, 2013; Tan et al., 2021) and these observations were
350 verified by our new data. But both the number of echemes and total sound produced also
351 indicated that their calling activity generally increases to a maximum in the afternoon before
352 decreasing towards dusk. This peak in calling activity is most obvious based on the total
353 sound produced. Likewise, for species within Lebinthini, our data further demonstrated with
354 greater precision that it may be too simplistic to broadly classify most species into either
355 nocturnal or diurnal species, since many species call at varying intensity across the circadian
356 cycle. *Lebinthus sanchezi*, *L. bitaeniatus* and *L. luae* calls during both day and night as
357 previously documented (Robillard & Tan, 2013; Robillard et al., 2013), but we further
358 showed that the calling activity undulates for these species. In the case of *M. jharnae*, the
359 species was previously documented as nocturnal: males and females signal to each other at
360 night and hide among leaf litters in the day (Robillard et al., 2016). Our data further
361 demonstrated that activity peaks slightly after midnight. Here, we also reported the circadian
362 rhythms of other species, i.e., *L. sandakan* and *C. admirabilis* for the first time. Even though
363 their calling songs were previously described (Tan & Robillard, 2014; Tan et al., 2019),
364 whether they are diurnal or nocturnal could not be confirmed.

365

366 Between the two *Cardiodactylus* species analysed here, *C. admirabilis* is strictly nocturnal,
367 but *C. palawan* calls in the day and slightly after dusk. Differing circadian rhythms of the call
368 activity within species of *Cardiodactylus* are expected, as field observations showed that
369 some species are clearly diurnal (e.g., *Cardiodactylus niugini*) or nocturnal (e.g.,
370 *Cardiodactylus muria* and *Cardiodactylus lombrinjani*) while others are more complex

371 (*Cardiodactylus sumba* which calls at night and mates in the day) (Robillard et al., 2014;
372 Dong & Robillard, 2016). Recordings and observations for more species in this speciose
373 genus are needed to quantify and characterise how lthe number of echemes and total sound
374 produced vary between species, and whether level of activity quantified by echemes and total
375 sound produced differ within each species.

376

377 Our recordings also indicated that both number of echemes and total sound produced can
378 offer different insights on the calling activity and circadian patterns. This is particularly true
379 for the *Nisitrus* and *Cardiodactylus*, while other eneopterines tend to show parallel variation
380 in the number of echemes and amount of sound produced across the circadian cycle.

381 Compared to other eneopterines, *Nisitrus* species produce an echeme sequence which is a
382 continuous trilling call over long periods of time, resembling the trilling calls of typical
383 gryllids. Hence, while they produce fewer calling bouts (i.e., echeme sequence) per hour,
384 they can still produce more sound per hour than other species.

385

386 On the other hand, the two species of *Cardiodactylus* produce many echemes, but produce a
387 shorter total duration of sound compared to other species. While other lebinthines generally
388 producing trilling echemes, this species produces few well-spaced syllables per echeme,
389 resulting in shorter duration of sound output. The *Nisitrus* and *Cardiodactylus* findings
390 highlighted that the eneopterines can utilise time differently, either by singing a few long
391 calls or many short calls of different densities.

392

393 Furthermore, *C. palawan* males showed two strong peaks in the number of echemes at 12:00
394 and 19:00 period. But this was not reflected based on the total sound produced, in which the

395 variations in the sound produced within active period are less pronounced. That *C. palawan*
396 can vary the number of syllables between three to 17 per echeme suggests that this species
397 may modulate the number of syllables (hence the sound produced per echeme) such that they
398 can either produce large number of shorter echemes and fewer number of longer echemes.
399 Such unusual behaviours are not commonly observed in crickets, and is worthy of further
400 investigation, especially since the *C. palawan* recorded in this study is limited in number.

401 ***Potential ecological drivers of circadian rhythm diversification***

402 Why different eneopterine species modulate their circadian rhythms differently can probably
403 be attributed to their ecology and behaviours (Yerushalmi & Green, 2009). Similar to
404 temporal call parameters, circadian rhythms are likely more labile and influenced by
405 behaviour and ecology and less constrained by sound-producing structures. Cyclical patterns
406 in the calling activity can be an adaptive form of predation avoidance by synchronising
407 calling with the inactive periods of predators and/or parasitoids (Greenfield, 1988; Fenn &
408 MacDonald, 1995). Recent study suggested that novel communication system in lebinthines
409 originated around the time of origin for bat echolocation (Benavides-Lopez et al., 2020),
410 further corroborating the idea that communication (which probably includes circadian
411 rhythms of calling activity) is strongly influenced by predation avoidance. Predator avoidance
412 can be particularly important for the lebinthines because the males call and explore
413 simultaneously to find mates (ter Hofstede et al., 2015), hence potentially exposing them to
414 greater risks of predation compared to typical gryllids. Since different eneopterine species
415 occupy different regions and different microhabitats (e.g., *Lebinthus*, *Microbinthus* and
416 *Macrobinthus* call on leaf litter or saplings nearer to the ground vs. *Cardiodactylus* and
417 *Nisitrus* which calls on foliage higher up in the plant), they may be exposed to different
418 predators specific in different regions within Southeast Asia and at different times of the day.

419

420 Both the two sister species, *L. luae* and *L. bitaeniatus*, have three peaks—in the dawn,
421 afternoon and night—in their circadian rhythms, but *L. luae* exhibits a strong drop in call
422 activity after the peak at dawn that was not observed in *L. bitaeniatus*. For the *L. luae* in
423 Singapore, it has been documented that this species is a major food source for the common
424 palm civet (*Paradoxurus musangus*) (Fung et al., 2018). These nocturnal-crepuscular
425 generalist omnivores are reported to be most active slightly before dawn and between dusk
426 and 21:00 (Chen et al., 2009; Fung, 2016; Zhao et al., 2020), which may explain the
427 distinctive drop in the call activity (with a very low variance) of *L. luae* around the same time
428 periods. However, such interpretations should be made cautiously because the circadian
429 rhythm of *L. luae* can be influenced by other predators and parasitoids with different
430 circadian rhythms that we are not aware of, as well as human disturbance (which is prominent
431 in city state of Singapore) (Tan & Robillard, 2021). Playback experiments using the sounds
432 of a range of potential predators and anthropogenic sound and light sources can reveal if and
433 how these factors impact circadian rhythms (Symes et al., 2020).

434

435 While some eneopterines call fairly consistently across their active period (e.g., *L. bitaeniatus*,
436 *L. sanchezi* and *M. jharnae*), other eneopterines calls intensely within short time periods,
437 sometimes one period per day (e.g., *L. sandakan*) and sometimes two periods (e.g., *C.*
438 *palawan* and *G. baitabagus*). High calling activity within short time period (i.e., 2–3 hours)
439 alternating with (near) absence of calling may also be another strategy to minimise their
440 exposure to predators and parasitoids. Similar strategies have been observed in other
441 orthopterans, including the *Gryllotalpa* species from Southeast Asia, which calls only in a

442 short time frame (~ 0.5 hours) around dusk, as well as in neotropical katydids (Tan &
443 Kamaruddin, 2013, 2014; Symes et al., 2020).

444

445 The circadian rhythm in the calling activity may also correspond tightly to the active period
446 of females, which also actively seek for males (Greenfield, 1988; 1992). This is particularly
447 relevant for lebinthines, because females produce vibrational replies and do not perform
448 phonotaxis, making the mate finding process more complicated (ter Hofstede et al., 2015)
449 compared to other orthopterans (e.g., Greenfield, 1992). Hence, it may be more energetically
450 conservative and less risky for males to call more often and search for females when the
451 females are more active rather than consistently across 24 hours. This will ensure that their
452 calls can draw the females' attention, ensuring higher mating opportunities while limiting the
453 risks of predation (Cade & Cade, 1992). This can be tested with more information about the
454 circadian rhythm of the activity level of females.

455

456 Lastly, the divergence in the circadian cycle can also be attributed to interspecific
457 competition for the acoustic niche, as observed in syntopic species of crickets from Southeast
458 Asia and neotropical katydids (Greenfield, 1988; Tan & Kamaruddin, 2013, 2014; Symes et
459 al., 2020; Tan, 2020). For example, in Singapore *C. admirabilis* and *N. malaya* are syntopic
460 (Fig. 5), but one is strictly nocturnal and the other is strictly diurnal. Likewise in Borneo
461 where *L. sandakan* and *N. vittatus* are syntopic (Fig. 5), we can observe that the peak of
462 activity for *L. sandakan* appears to correspond with a dip in activity for *N. vittatus*. That the
463 *Nisitrus* species produce long echeme sequences may interfere the short echemes produced
464 by the respective syntopic eneopterines.

465

466 On the other hand, we do not see a distinct diel partitioning for other sympatric species pairs
467 of *L. bitaeniatus* and *L. sanchezi* in the Philippines, and *L. luae* and *N. malaya* in Singapore
468 (Fig. 5). In the case of the two *Lebinthus* species from the Philippines, they are not fully
469 sympatric as a result of occupying slightly different habitats (i.e., forested and open areas).
470 Furthermore, clear-cut diel partitioning among sympatric eneopterine species can be non-
471 existent because these crickets also face masking interference from other orthopterans and
472 sound-producing insects, rather than merely their close relatives (see Diwakar &
473 Balakrishnan, 2007; Jain et al., 2014). Furthermore, sound-producing insects can also
474 partition the acoustic niche by the combination of call structure, intensity and frequency
475 (Diwakar & Balakrishnan, 2007; Jain et al., 2014). Hence, masking interference from
476 sympatric species may not exert such a strong selection pressure on the circadian cycle as
477 compared to predation risk or environmental noises (Greenfield, 1988; Jain et al., 2014).

478

479 ***Conclusions***

480 In conclusion, while we have presented data for all but three genera of Southeast Asian
481 eneopterines (Fig. 4), our current knowledge and new data do not yet allow us to pinpoint the
482 precise origin of the diversity of circadian rhythms observed in Eneopterinae from Southeast
483 Asia. Crucially, we were not able to record Southeast Asian species from the Xenogryllini
484 tribe (Fig. 4). Broader taxonomic sampling of speciose genera (e.g., *Cardiodactylus*) is also
485 needed. Furthermore, it should also be noted that the crickets used in this study are likely to
486 be entrained by their neighbours in the community tank or natural habitats and by
487 environmental zeitgebers prior to recording. These confounding factors can have a more
488 critical effect for wild-collected individuals than lab colonies. As such, one should either (i)
489 record both wild-collected individuals and lab colonies or (ii) increase sample size to control

490 for such effects. Otherwise, it is necessary to exercise caution in future comparative analyses
491 involving data collected from both wild-collected individuals and lab colonies.

492

493 Nonetheless, we can postulate that the diversity in the circadian rhythms is influenced by a
494 combination of factors: avoiding the active period of predators and parasitoids as well as
495 maximizing the overlap with the females' preference and active period. Multiple parameters
496 of the circadian calling cycle may evolve dynamically, with changes in the duration of the
497 active window as well as diurnal *vs.* nocturnal calling. Historically, circadian rhythm has
498 been overlooked in communication studies related to both sexual and natural selection
499 pressures. If shifts in the dominant frequency of songs is attributed to predator avoidance
500 (Robillard & Desutter-Grandcolas, 2011a; Robillard et al., 2013), the same could be
501 happening for circadian rhythms. Incorporating information about the circadian rhythms will
502 reveal that the evolutionary story of the communication in eneopterines can be more complex
503 than currently understood. For many of the Southeast Asian species, little is known about
504 their ecology and natural history; so while our results are hitherto descriptive, they can still
505 offer opportunities to question the roles of circadian rhythms in the complex communication
506 system and its evolution.

507

508

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517

518

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708

709

710 **Figure captions**

711

712 Figure 1. Oscillograms of the calls of the 11 species of Eneopterinae. The red box represents
713 one echeme.

714

715 Figure 2. The circadian rhythms in the number of echemes produced by 11 species of
716 Eneopterinae. Smoothed lines were fitted using generalised additive mixed effects models
717 with quasipoisson error structure; red lines and 95% CI represent diurnal species, blue lines
718 represent nocturnal species, green lines represents complex species, black lines represent
719 species with unclear/ no pattern. The grey area indicates dark hours; white areas indicate light
720 hours. N = number of males recorded.

721

722 Figure 3. The circadian rhythms in the sound production (s) for 11 species of Eneopterinae.
723 Smoothed lines were fitted using generalised additive mixed effects models with gaussian
724 error structure; red lines and 95% CI represent diurnal species, blue lines represent nocturnal
725 species, green lines represent complex species, black lines represent species with unclear/ no
726 pattern. The grey area indicates dark hours; white areas indicates light hours. N = number of
727 males recorded.

728

729 Figure 4. Simplified phylogeny of Eneopterinae based on Vicente et al. (2017). Focal taxa are
730 indicated in bold with symbols representing the circadian pattern. Red circles represent
731 diurnal species, blue squares represent nocturnal species, green circles and squares represent
732 diurnal and nocturnal peaks of species with complex circadian patterns, black circles
733 represent species with unclear/ no pattern. The number of circles/ squares for each species

734 represents the number of observable peaks in their circadian rhythms. Clades in yellow
 735 represent genera that occur in Southeast Asia, grey clades represent genera or tribes that
 736 occur outside Southeast Asia.

737

738 Figure 5. Map of Southeast Asia showing the estimated distribution of the 11 eneopterine
 739 species. Black dots represent the locations where individuals were collected to initiate the
 740 colonies recorded in this study.

741

742 Table 1. List of species studied with their location of origin, the number of males that were
 743 recorded, and the number of males that sang. Not every focal male sang during the 24-hr
 744 recording period, and the number of individuals which were recorded and sang were
 745 distinguished here.

Species	Origin	#♂ recorded	#♂ sang
Lab colonies			
<i>Nisitrus malaya</i>	Malay Peninsula	15	9
<i>Nisitrus vittatus</i>	Borneo	10	8
<i>Cardiodactylus palawan</i>	Philippines	5	5
<i>Macrobinthus jharnae</i>	New Guinea	16	14
<i>Microbinthus pintaudi</i>	New Guinea	21	18
<i>Gnominthus baitabagus</i>	New Guinea	3	3
<i>Lebinthus (sanchezi species group) sanchezi</i>	Philippines	19	12
<i>Lebinthus (sanchezi species group) sandakan</i>	Borneo	20	19
<i>Lebinthus (bitaeniatus species group)</i>	Philippines	19	17

bitaeniatus

Wild individuals

<i>Cardiodactylus admirabilis</i>	Singapore	5	5
<i>Lebinthus (bitaeniatus species group) luae</i>	Singapore	30	27
Total		163	137

746

747 Table 2. Summary descriptions of the circadian rhythm of the calling activity in the 11

748 eneopterine cricket species. The number of hours in which the cricket was active was

749 specified for species that do not call throughout the circadian cycle. The time of the peak(s) is

750 also specified.

Tribe	Species	Activity period	Activity peaks
Nisitritini	<i>Nisitrus malaya</i>	Diurnal, ca. 15 hrs	One diurnal peak in the afternoon
Nisitritini	<i>Nisitrus vittatus</i>	Diurnal, ca. 13 hrs	One (or two) diurnal peaks in the afternoon
Lebinthini	<i>Cardiodactylus admirabilis</i>	Nocturnal, ca. 10 hrs	One nocturnal peak from late evening to midnight
Lebinthini	<i>Cardiodactylus palawan</i>	Complex	Two peaks: diurnal (around noon), nocturnal (after dusk) (by echeme); consistent (by sound)
Lebinthini	<i>Macrobinthus jharnae</i>	Nocturnal, ca. 13 hrs	One nocturnal peak around midnight

Lebinthini	<i>Microbinthus pintaudi</i>	Complex	Consistent
Lebinthini	<i>Gnominthus baitabagus</i>	Complex, narrow	Two peaks: diurnal (afternoon), nocturnal (midnight)
Lebinthini	<i>Lebinthus sanchezi</i>	Complex, undulating	Two weak peaks: dawn, nocturnal (late evening)
Lebinthini	<i>Lebinthus sandakan</i>	Diurnal, ca. 6 hrs	One diurnal peak before noon
Lebinthini	<i>Lebinthus bitaeniatus</i>	Complex, undulating	Three weak peaks: dawn, diurnal (early afternoon), nocturnal (late evening)
Lebinthini	<i>Lebinthus luae</i>	Complex, undulating	Three weak peaks: dawn, diurnal (early afternoon), nocturnal (after midnight)