Cicada timetree by BEAST v1.X verified the recently and exponentially increasing base substitution rates

Soichi Osozawa 1*, and John Wakabayashi 2

1 KawaOso Molecular Bio-Geology Institute, Sendai, 982-0807, Japan,
Former: Institute of Geology and Paleontology, Faculty of Science, Tohoku
University

2 Department of Earth and Environmental Sciences, California State
University, Fresno, CA 93740

*Corresponding author. E-mails: kawaoso@icloud.com (S. Osozawa)

Abstract

Following the recent publication of global cicada phylogenetic trees by Marshall et al. (2018), Łukasik et al. (2018), and Simon et al. (2019), we developed a new dated tree incorporating mostly endemic east Asian cicada data for totally 113 specimens, using the mostly advanced BEAST v1.X software applied the relaxed clock model. Fossil calibrations as old as Triassic were adopted after Moulds (2018), and a Quaternary geological event calibration was adopted following Osozawa et al. (2012), applying the calibration function of BEAST. Our timetree suggests that Tettigarctidae had cicada basal lineage as old as 200 Ma, and Derotettiginae was next as

old as 100 Ma. Tibicininae was a sister of the resting Cicadidae, and Tettigomyiinae, Cicadettinae, and Cicadina started simultaneous branching and radiation around 40 Ma. We made a base substitution rate vs age diagram based on the timetree using the BEAST function, and it strongly suggested an exponential increase of base substitution rate approaching the present. The consequent increased cicada biodiversity including generation of cryptic species might have been driven by the generation and spreading of C4 grasses and the following Quaternary glaciations and severe environmental change.

Key words: fossil calibration, geological event calibration, exponentially increased base substitution rate, increased biodiversity, cryptic species, ice age, C4 grasses

Introduction

A phylogenetic tree of worldwide cicada was recently constructed by Marshall et al. (2018) and Simon et al. (2019), applying five concatenated sequences of mitochondrial COI and COII, and nuclear ARD1, EF-1a, and 18S rRNA, and by Łukasik et al. (2019), applying whole mitochondrial sequences for representative species.

These Simon group studies included comparatively little Asian data. After Osozawa et al. (2017), which treated *Platypleura* endemic cicadas in

east Asia, mostly from the Japan, Ryukyu, and Taiwan islands, we collected cicada samples and increased the available DNA sequence data of mitochondrial COI and nuclear 18S rRNA (Table 1; totally 92 specimen data / $92 \times 2 = 184$ sequence data) were newly added to the already published 70 specimen data of *Platypleura* (duplicated sequence data were eliminated, and totally 21 specimen data were considered to construct the present timetree). Our east Asian data combined with the global data offered by Marshall et al. (2018; including 20 east Asian species / totally 149 species in their figure 4) and Łukasik et al. (2018; including 27 east Asia species / totally 93 species in their figure 2) allows the construction of a more complete phylogenetic tree, and the operational taxonomic units (OTUs) including outgroup species are 191 for the COI timetree in Figs. 1 and 2, and 155 for the COI +18S rRNA timetree in Fig. 3. Note that the COI and 18S rRNA data in Table 1 by Marshall et al. (2018) contain many missing and incompatible data, and their partial 42 data / total 149 data (our own data: 113) were only available in our analyses.

Phylogenetic trees by Marshall et al. (2018) and Łukasik et al. (2018) were not dated phylogenies, whereas the latest version of BEAST (Bayesian Evolutionary Analysis Sampling Trees; Suchard et al., 2018) v1.X released on 10th June 2018 has a fossil age calibration protocol. This calibration involves applying times of the most recent common ancestors (tMRCAs) of the ingroup species in the associated software of BEAUti (Bayesian Evolutionary Analysis Utility). Minimum and maximum age constraints in

MCMCtree is complexly defined (Benton & Donoghue, 2007; Marshall, 2008), and application of the latter is not easy, but it is not needed in BEAST v.1.X. Moulds (2018), one of authors of Marshall et al. (2018), reviewed the ages of cicada fossils, and these dates up to 200 Ma were available for our fossil-based time calibrations in BEAUti. As shown by Osozawa et al. (2017), *Platypleura* and some other cicadas can be additionally and simultaneously calibrated by the Quaternary geological event calibration at 1.55 Ma. In addition, partitioning in BEAUti is performed simply by applying each gene sequence, not by concatenated sequences found in all the previous studies including Marshall et al. (2018). Using this most recent version of BEAST (v1.X), we can build an accurate and reliable cicada timetree.

Through these analyses, we corroborate the classification and some rearrangement of species into four subfamilies of Tibicininae, Tettigomyiinae, Cicadettinae, and Cicadinae included in a family of Cicadidae by Marshall et al. (2018) and Łukasik et al. (2018), and then estimate the crown dates of these subfamilies and tribes, and the dates of every species (Fig. 1). In the present analyses, we include *Derotettix*, a relict species of new subfamily of Derotettiginae with the oldest lineage in Cicadidae (Simon et al. 2019), and also try to estimate the crown date. Comparison in the context of the whole Hemipteroid insect timetree (Johnson et al., 2018) and whole insect timetree (Misof et al. 2014) can be conducted as an extension of this analysis, adding other Hemiptera species

93 as outgroup.

94 Our main purpose was to represent precise evolutionary history of whole
 95 cicadas by contracting the BEAST timetree, but we have additional bellow
 96 because we have new attractive finding. Recent phylogenetic analyses
 97 usually employs relaxed clock model, which allows each branch of a
 98 phylogenetic tree to have its own evolutionary rate (Drummond et al. 2012).
 99 Although the relaxed distribution can be set such as lognormal in BEAUti,
 100 the rate of variability has not been considered till the present study.
 101 Because the output figure of BEAST v.1X represents the base substitution
 102 rate and age at each node, we can check the acceleration or delay of rate
 103 through the time on the newly built timetree, as the first trial. Chronogram
 104 by Marshall et al. (2016) for Australian Cicadettini might be unsuccessful,
 105 probably because of unsuitable calibration method and software choice.

106 In our previous experience of *Platypleura* cicadas calibrated solely by
 107 1.55 Ma (Osozawa et al. 2017), our outgroup choice of *Tettigarcta crinita*
 108 output the extremely young origin as old as 10 m.y. We can now explain
 109 that the one digit slow base substitution rate of old time than the present
 110 affected on the apparently young date of the *Tettigarcta* lineage.

111 We show that the increasing cicada diversity reflects exponentially
 112 increasing base substitution rate approaching recent times (Figs. 1 ~ 3
 113 inset), that may be a consequence of extensive adaptive radiation triggered
 114 by the start of the Quaternary glacial-interglacial cycles and severe
 115 environmental and climatic change. Although Tettigarctidae is a living

fossil as old as 200 Ma, our work shows that the Cicadinae subfamily differentiation mostly took place after 40 Ma, and the speciation is relatively younger event. The latter is partly a consequence of the Quaternary vicariant speciation of East Asian cicada in the Ryukyu and Taiwan region (e.g., Osozawa et al. 2017), but rather expresses the increased biodiversity reflecting the recently accelerated base substitution rate and speciation including the cryptic species found in each island or island group.

Materials and Methods

Taxon sampling and applied DNA sequence

COI and 18S rRNA sequence data from our collected 113 specimens are in Table 1. Primers used, amplifications, and sequencing are given in Osozawa et al. (2017). These sequences were aligned by ClustalW in MEGA X (Kumar et al. 2016). The COI sequence data comprise 1,534 bp, and the 18S rRNA sequence 874 bp, and the resolution to construct phylogenetic tree is enough, as we experienced in the previous *Platypleura* paper (Osozawa et al. 2017). The COI data in Marshall et al. (2018) comprised 1485 pb comparable to ours, and their COII data comprised 684 pb. Nuclear 18S rRNA represented less variation with much slower base substitution rate compared to mitochondrial COI, and the topology was unaffected (Osozawa et al. 2017). North American Cryptotympanini was analyzed by Hill et al.

(2015), applying 1467 bp of COI and 783 bp of nuclear EF-1a, and the resolution was enough. Mostly Australian Cicadettini was analyzed by Marshall et al. (2016), applying 1492 bp of COI and 1047 bp of nuclear EF-1a, and the resolution was also enough.

We incorporated other representative cicada data from GenBank / DDJB, derived from Marshall et al. (2018) and Łukasik et al. (2019), and included these data in our BEAST analyses. Note that Tettigarctinae, Derotettiginae, Tibicininae, and Tettigomyiinae are not known from East Asia, and Cicadettinae has only two species of *Kosemia* in Japan main islands. By this restriction, our own worldwide taxon sampling was very difficult, and GenBank / DDJB data offered by Marshall et al. (2018) and Łukasik et al. (2019) were essential for us. Whole mitochondrial sequence data by Łukasik et al. (2019) are included in our analyses as corresponding COI regions. However, note that not all data found in Marshall et al. (2018) corresponded to our COI and 18S rRNA sequences, and these data were not available in our analyses. Within COI sequence data in Marshall et al. (2018), only 21 data for Cicadettinae and only 14 data for Cicadinae are available in our analyses even by considering data found in Łukasik et al. (2019). 18S rRNA sequence in Marshall et al. (2018) is only available for *Nablistes heterochroma* of Tettigomyiinae and *Platypedia putnami* of Tibicininae. Marshall et al. (2018) additionally offered sequence data of COII, ARD1, and EF-1a, but these data in their table 1 were not enough to cover all their OTUs on figure 4. In the first step, we made a timetree only applying COI

sequence data (Figs. 1 and 2; 191 OTUs) that covers Tettigomyiinae and Tibicininae species. Next, we made a timetree generated by applying both COI and 18S rRNA sequences (Fig. 3; 155 OTUs). We confirmed that our analyses with or without 18S rRNA data did not affect on the topologies.

In our analyses, single OUT did not represent single species (Table 1). *Platypleura* cicada (Osozawa et al. 2017) was experienced severe vicariance affected by the 1.55 isolation of the Ryukyu, Japan, and Taiwan islands from Chinese continent (Osozawa et al. 2012), and we needed to collect each island population for each *Platypleura* species. Similarly, we newly collected cicada specimens in this paper from each island population of *Mogannia* (Cicadettinae), and *Cryptotympana*, *Graptopsaltria*, *Hyalessa*, *Pomponia*, *Meimuna*, *Tanna*, and *Euterpnosia* (Cicadinae). *Hyalessa maculaticollis* was affected by vicariance within China and Japan (Liu et al. 2018). As noted above, Tettigarctinae, Derotettiginae, Tibicininae, and Tettigomyiinae are lacking in East Asia, and we could not collect these cicadas.

Phylogenetic analyses associated with fossil and geological event calibrations by BEAST v1.X

A Bayesian inference (BI) tree (Figs. 1 ~ 3) was constructed using the software BEAST v.1X, running BEAUti, BEAST, TreeAnnotator, and FigTree, in ascending order. Before operating the BEAST software, the BEAGLE Library must be downloaded. Tracer v.1.6 was applied for

checking the calculation status and estimating such as mean base substitution rate.

For graphic explanation of the operation of this software, see the “BEAST operating manual” at:

<http://kawaosombgi.livedoor.blog/archives/11386037.html>

In BEAUti, “Taxa” and “Priors” (date setting is in “Prior”; date should be set in box of “tMRCA”) were set as follows for ingroup species relative to two partitions of COI and 18S rRNA. These partitions automatically appear in “Partitions” without traditional PartitionFinder, if these sequence data are uploaded by using the plus button or by “Import Data”.

Calibrations points are shown on Figs. 1 and 2, and these dates were input in “Priors” in BEAUti; they are summarized below. Corresponding ingroup species were included in ingroup taxa by “Taxon Set” on the “Taxa” screen in BEAUti. Other than calibration points A1 to A6 for the outgroup (Fig. 1), the fossil calibrations are after Moulds (2018). For the latter, some are based on radio-isotopic dating the fossil-bearing strata, whereas others are based on biostratigraphy assigned to an age/stage on the geologic time scale, for which absolute age ranges are generally based on radio-isotopic dates of associated strata in key global localities. This time scale has been standardized by the International Commission on Stratigraphy (ICS) (www.stratigraphy.org) and the most recent version of the time scale is available at <http://www.stratigraphy.org/index.php/ics-chart-timescale>, and the explanatory paper related to the generation of the time scale is Cohen *et*

al. (2013). Calibration points Q1 to Q12 are after our geological event calibration that adopts a 1.55 Ma date based on multiple biostratigraphic and radio-isotopic dates connected to various geologic relationships in the Ryukyu islands region (Osozawa *et al.* 2012): this geologic event calibration was used in a previous study of *Platyleura cicadas* by our group (Osozawa *et al.* 2017).

The shape of the underlying chronogram was controlled and affected by the calibration points and dates in the BEAUti setting as shown by prior age (input) in Figs. 1 ~ 3, and in this meaning the BEAST calibration was “active” one. If the shape was unreasonable including a case of output age was distinct from input age, we reset the calibration and repeatedly run BEAST until to get reasonable tree. We show below the final calibration points and their dates.

The specific calibration points and dates used are as follows:

Calibration point A1: Crown Hemiptera: Fossil Aphidoidea was reported from the French Bundsandstein (Szwedo and Nel 2010; Bashkuev *et al.* 2012) of Anisian age (244.5 ± 2.5 Ma).

A2: The oldest fossil Belostomatidae was reported from the Zagaje Formation, Poland (Popov 1996) of Hettangian age (200.3 ± 1.0 Ma).

A3: Fossil Cicadellidae including Ledridae (Zhang 1997) and fossil Cercopidae (Hong 1982) was from the Jehol Biota, northern China. The Jehol Biota horizon has been dated by the Ar-Ar method on associated silicic tuff at 130.7 ± 1.4 Ma (He *et al.* 2006).

231 A4: Fossil Gerridae is from French amber (Perrichot et al. 2005) of Albian
232 age (107 ± 6 Ma).

233 A5: Fossil Delphacidae is from the Green River Formation, USA (Grande
234 1980). Ar-Ar dating on silicic tuff within the formation yields ages of 53.5 –
235 48.5 Ma (weighted average age of 51.25 ± 0.31 Ma; Smith et al. 2003).

236 A6: Fossil Flatidae was from the Maíz Gordo Formation, northwest
237 Argentina (Petrulevičius 2011) of Paleocene age (61 ± 5) Ma.

238 Calibration point B: Crown Tettigarctinae: Fossil Tettigarctinae was
239 found in strata Dorset, England (Whalley 1985) of Hettangian age (203.1 ± 1.0
240 Ma).

241 Calibration point C: Stem Derotettiginae: Food plant of *Derotettix*
242 *mendosensis* is Amaranthaceae in Argentina (Simon et al., 2019), and this
243 worldwide C₄ plant was phylogenetically studied by Piirainen et al. (2017).
244 This fossil was reported by Zucol et al. (2018), and the fossil horizon was
245 dated by Ar-Ar method at 49.512 ± 0.019 Ma (Eocene; Woodburne et al.,
246 2014). However, fossil *Burmacicada protera* was found from Burmese amber
247 (Poinar and Kritsky 2011), and the zircon U-Pb age of the amber bearing
248 matrix was 98.79 ± 0.62 Ma (Shi et al. 2012). We applied this date for stem
249 Cicadidae and stem Derotettiginae.

250 Calibration point D: Stem *Platypedia putnami* (Tibicininae): Fossil
251 *Platypedia primigenia* was found in the Florissant Formation, Colorado,
252 USA, and associated strata was dated by Ar-Ar method at 35.15 ± 1.65 Ma
253 (McIntosh et al. 1992). However, we used older date of crown Tibicininae:

254 Fossil *Davisia bearcreekensis* was found in the Upper Fort Union
255 Formation, Montana, USA (Cooper 1941), and the age was considered the
256 Thanetian of 57.6 ± 1.6 Ma (Flores and Bader 1999).

257 Crown Cryptotympanini: Fossil *Hadoa grandiose* was also found in the
258 Florissant Formation, Colorado, USA, and associated strata was dated by
259 Ar-Ar method at 35.15 ± 1.65 Ma (Mcintosh et al. 1992), but this calibration
260 generated unreasonable tree.

261 Calibration point E: Crown Cicadettinae: *Paracicadetta oligocenica*
262 (Boulard and Nel 1990) was from Cereste, France, and this famous fossil
263 locality was considered to be the Rupelian of 31 ± 2.9 Ma.

264 Calibration point F: Stem *Lyristes plebejus*: Fossil *Lyristes* sp. was
265 reported from Seifhennersdorf, Germany, and associated strata was dated
266 by the K-Ar method as 30.44 ± 1.52 Ma (Walther and Kvacek 2007).

267 Calibration point G: Crown *Cryptotympana*: Fossil *Cryptotympana*
268 *incasa* and *C. miocenica* (G1), and also *Hyalessa lapidescens* (G2) were
269 found from Shanwang, Shandong, China, and these strata are considered to
270 be time correlative to the European MN5 mammalian unit (16.45 ± 0.45 Ma;
271 Roc̆ek et al. 2011).

272 Calibration point H: Crown *Meimuna opalifera*: Fossil *Meimuna*
273 *protopalifera* was found from the Itamuro Formation, Tochigi, Japan
274 (Fujiyama 1982; Yoshikawa 2005), and the fission track age of the
275 correlative terrestrial strata of the Nashino Formation, Sendai, is 6.4 ± 0.4
276 Ma (Fujiwara et al. 2008).

Calibration point Q: The dates of the common ancestors of *Mogannia*
minuta (Q1), *M. hebes* (Q2), *Cryptotympana facialis* (Q3), dark winged
Platypleura (Q4), right winged *Platypleura* (Q5; Osozawa et al. 2017),
Graptopsaltria nigrofuscata + *G. bimaculata* (Q6), *Meimuna opalifera* (Q7),
Meimuna kuroiwae (Q8), *M. oshimaensis* + *M. iwasakii* + *M. goshizana*
(Q9), *Tanna japonensis* + *T. japonensis ishigakiana* + *T. sozanensis* + *T. sp.*
(Q10) *Euterpnosia chibensis* + *E. chibensis daitoensis* + *E. chibensis*
okinawana (Q11), *E. iwasakii* + *E. viridifrons* + *E. olivacea* + *E. gina* + *E. sp.*
(Q12): The date assigned is that of a geological event, which is of isolation of
the Ryukyu islands from the Chinese continent by the opening of the
Okinawa trough that began (i.e., islands had separated from mainland and
each other by this time) at 1.55 ± 0.15 Ma (Osozawa et al. 2012). The age
assignment is from multiple biostratigraphic and radio-isotopic ages from
the oldest marine strata on the landward side of the islands as well as the
sides facing other islands, so that the age of such strata constrain the
physical separation of the islands from the mainland and each other. Note
that no land bridge for dispersal was geologically evidenced in the Ryukyu
islands.

Results

Hemiptera Timetree (Fig. 1)

Because topology is concordant between Figs. 1 and 2 (COI) and Fig. 3 (COI

+ 16S rRNA), we describe bellow based on Fig. 2 with 191 OTUs, although each posterior age (output) was not all the same. Based on our analyses, the subfamily-group classification follows Marshall *et al.* (2018), Łukasik *et al.* (2019), and Simon *et al.* (2019).

Hemiptera, including Cicadoidea, has a single common ancestor of 243.11 Ma, as calibrated by the 244.5 ± 2.5 Ma age reviewed above. Phylogeny of the outgroup Hemiptera calibrated by A1 to A6 was concordant to Johnson *et al.* (2018) and Misof *et al.* (2014).

In the Cicadoidea ingroup, Tettigarctidae was an old lineage differentiated from Cicadidae at 200.25 Ma, as calibrated by 200.3 ± 1 Ma, so Tettigarctidae is essentially a living fossil that have persisted since ca. 200 Ma. We estimated a date of the common ancestor of two extant species of *Tettigarcta tomentosa* (Tasmania) and *T. crinita* (southeast Australia) at 15.05 Ma, and the youngest fossil of Tettigarctinae was reported from the Aquitanian (21.735 ± 1.295 Ma), southern New Zealand (Kaulfuss & Moulds, 2015). However, Tettigarctidae includes 19 extinct genera according to Kaulfuss¹ and Moulds (2015), whereas modified as many more according to Moulds (2018).

Simon *et al.* (2019) proposed a new subfamily Derotettiginae consisting of a single species of *Derotettix mendosensis*, which is a sister of resting Cicadidae species and the oldest lineage species in Cicadidae dated at 98.85 Ma. Łukasik *et al.* (2018) already showed such the phylogenetic position of *D. mendosensis*.

Our timetree showed that Tibicininae is a sister of Tettigomyiinae + Cicadettinae + Cicadinae differentiated at 65.89 Ma, and Tibicininae started differentiation at 57.07 Ma. Tettigomyiinae is a sister of Cicadettinae differentiated at 35.67 Ma, Tettigomyiinae + Cicadettinae is a sister of Cicadinae differentiated at 39.44 Ma. Cicadettinae started differentiation at 31.48 Ma, Cicadinae started differentiation at 36.7 Ma. Differentiation of Tettigomyiinae + Cicadettinae took place simultaneously after 39.44 Ma.

A single common ancestor of Cicadidae excepting Derotettiginae started differentiation and speciation into Tibicininae, Tettigomyiinae, Cicadettinae, and Cicadinae at 65.89 Ma. Although the pre-Miocene fossil Cicadidae collectively include ten extinct genera, comprising *Davisia* and *Lithocicada* for Tibicininae, *Paracicadetta*, *Paleopsalta*, *Minyscapheus*, and *Miocenoprasia* for Cicadettinae, and *Burmacicada*, *Camuracicada*, *Tymocicada*, *Dominicicada* for Cicadinae, the remaining 23 genera post-Oligocene fossil cicadas are extant (Moulds 2018). Cicadidae, conflict to the fossil record, consisted of only one species but coexisted with a Tettigarctidae species between 200.25 and 65.89 Ma, and cicada biodiversity was extremely low during this period excepting for extinct species and *D. mendosensis*.

The geologic calibration point Q1 to Q12 at 1.55 ± 0.15 Ma applies to multi furcations that were recognized for *Mogannia minuta* and other cicadas endemic to in the Ryukyu islands and Taiwan as noted above. Each island or island group population was mostly genetically distinct, endemic,

and criptic, as shown for *Platycleura* in Osozawa et al. (2017).

Inconsistent cicada base substitution rate (Figs. 1 ~3 insets)

Comparing base substitution rate vs age shows that the rate has not been constant; the rate appears to have exponentially increased approaching the present. The data points, fitted trendline, and associated equation are shown on the insets of Figs. 1 and 2. The trendlines and associated rates are similar for analyses based on COI alone (Figs. 1 and 2), and combined COI + 18S rRNA (Fig. 3).

Discussion

Recently increased cicada biodiversity

Hemipteroid insects of Psocodea, Thysanoptera, and the subject of this study, Hemiptera, include 120,000 described species which comprise over 10% of known insect diversity, date back to 400 Ma (Hemiptera: 300 Ma; Johnson et al. 2018). Johnson et al. (2018) estimated primarily Cretaceous dates of differentiation into species, including Cercopoidea, Gerriidea, Flatidae, and Cicadoidea, that are common to our analyses, although they analyzed only two to nine superfamily taxa, in contrast to the 180 taxa of Cicadoidea and 8 taxa for other Hemiptera of our analyses. Misof et al. (2014) estimated mostly in pre-Paleogene dates of differentiation into

species including Cercopoidea, Aphididae, and Delphacidae common to our analyses, with less than 13 taxa analyzed. Their higher level phylogeny suggested long branches and old lineage of each super family species concordant to ours, and did not suggest the geologically recent increase in insect diversity apparent from our analyses of 191 Hemiptera taxa.

In Fig. 2, ingroup Cicadoidea, excluding Derotettiginae, underwent extensive differentiation into 180 taxa after 65.89 Ma, mostly after 39.44 Ma, leading to increasing biodiversity of Cicadoidea. Cicadoidea consisted of only two species excluding *D. mendosensis* between 98.85 and 65.89 Ma, although Cicadoide contains many extinct species that remain to be identified as fossils (Moulds 2018).

Increased biodiversity was typically presented by cryptic species in each island of Ryukyu. For example, *Platypleura kaempferi* in the Amami and Okinawa islands has light colored wings contrasting to that in Japan-Korea-China and Taiwan with dark colored wings, and the clade was distinct each other (Osozawa et al., 2017). *P. kaempferi* is not a single species but includes at least two cryptic species of light or dark winged *Platypleura*. Cicadas calibrated by other Q points included cryptic species, which also contributed increasing biodiversity. The Okinawa trough is spreading now, the Ryukyu islands are separating from the Chinese continent, and the vicariantly speciation and radiation is under progress, which is also contributing increasing biodiversity. In China mainland, *Hyalessa maculaticollis* and *Platypleura hilpa* were extensively radiated to form

cryptic species (Liu et al., 2018, 2020).

Exponentially increased base substitution rate as a factor of Hemiptera diversity, and their possible causes

Figs. 1 ~ 3 insets show a large range of base substitution rates for different time periods, at variance with the constant molecular clock hypothesis (relatively constant rate over time; Ho 2008). The trend in base substitution rates shows an exponential increase approaching recent time.

Such an increase in base substitution rate was first shown for taxa such as primates by Ho et al. (2005) who showed that a Quaternary calibration date resulted in a more a rapid base substitution rate than that associated an older calibration date. They employed an older version of BEAST (v1. 3; Drummond and Rambaut, 2003) that required repeated runs, applying a date at each calibration point. In contrast, BEAST v1.X, used in our analyses, can simultaneously apply multiple calibration points, as we have done using dates ranging from the Triassic to the Quaternary. As a result, the calculated increasing rate of base substitution in our analyses is not an artifact of a Quaternary calibration, but is constrained by multiple age calibrations across a wide range of geologic time. Therefore, although the base substitution rate trendlines and associated equations of Ho et al. (2005) are similar to ours, their timetrees do not reflect the changing of base substitution rates through time, but rather reflect a constant base

substitution rate as if constrained by a strict molecular clock. A similar situation relative to our BEAST analyses was for beetles in the Aegean region reported by Papadopoulou et al. (2010).

The increasing base substitution rate is apparently associated with the recently increasing cicada diversity, expansion, and radiation (in Fig. 2 timetree) that started at 39.44 Ma. The timing of the most rapid diversification coincides with Quaternary environmental change, marked by the start of glacial-inter glacial cycles. The initiation of Quaternary glaciations may have been triggered by rapid expansion of land grasses (Poales), that led to increased carbon fixation that decreased atmospheric CO₂ concentrations, because of the high efficiency of CO₂ fixation of such C₄ plants (Taira, 2007; Sage, 2004). C₄ Poales appeared and began diversification during the Oligocene (23 – 33.9 Ma) based on molecular clock approach, and after 14.5 Ma based on fossil evidences (Sage 2004). We estimated 15.58 Ma by our Angiospermae timetree, also employed BEAST v1.X with robust plant fossil calibrations (Osozawa et al., 2020).

Feeding plants of *D. mendosensis* are, however, C₄ dicots of Amaranthaceae (see figure 9 in Sage 2004) and Chenopodiaceae in degraded salt-plain habitats in arid regions of central Argentina (Simon et al. 2019). These dicot fossils but including C₄ monocot fossils of Poales grass (Chloridoideae) were reported from the Eocene in Patagonia by Zucol et al. (2018), **and** the fossil horizon was dated by the Ar-Ar method of 49.512 ± 0.019 Ma (Woodburne et al. 2014). The C₄ photosynthetic pathway began

from 50 Ma in South America, earlier than elsewhere. As for Chloridoideae, however, transition from C3 to C4 photosynthesis occurred in the Oligocene (23~33.9 Ma) was reported by Christin et al. (2008), but Sage (2004) suggested the fossil date 14.5 Ma similar to the above.

The ultimate factor of increasing biodiversity was thus generation and radiation of C4 plants and development of grass lands on the Earth since the Oligocene but probably middle Miocene, through the decreasing atmospheric CO2 concentrations, start of the Quaternary ice ages, adaptive radiations, and increasing base substitution rates. The Earth environment was thus much affected by life activity including C4 grasses, and if no life, the Earth might have become the completely different bleak planet.

Declaration of competing interest

The authors declare that they have no conflict of interest.

Supplementary Material

Supplementary data in Table 1 are available at GenBank / DDBJ.

Data Availability Statement

All relevant data are within the manuscript.

Acknowledgements

We thank Chris Simon and David Marshall for privately offering

Tettigarcta sequence data (later released in GenBank / DDBJ). We thank the collectors shown in Table 1. Bor-ming Jahn (Taiwan University; deceased 1 December, 2016), Ping-Shih Yang (Taiwan University), Chin-Ho Tsai (National Dong Hwa University), and Jen-Zon Ho and Hua-Te Fang (Endemic Species Research Institute) supported sample collections and obtained permission to collect in Taiwan.

Funding

This project was partly financed through the Osozawa Fund (Former), Tohoku University. We thank Keiji Nunohara (Nunohara Office for Geological Survey), Kohei Sugawara (Ecofarm GSK), CTI Engineering Co., Ltd., and NEWJEC, Inc. for contributing to this fund.

Author Contributions

Soichi Osozawa collected samples and coordinated the research, carried out the DNA analyses, and wrote this paper, and John Wakabayashi (native English-speaking American geologist) edited the writing.

References

- Benton MJ, Donoghue PCJ. 2007. Paleontological evidence to date the tree of life. *Molecular Biology and Evolution* 24: 26–53.
- Benton MJ, Donoghue PCJ, Asher RJ, Friedman MN, Thomas J, Jakob V. 2015. Constraints on the timescale of animal evolutionary history.

- 484 *Palaeontologia Electronica* 18.1.1FC: 1-106.
- 485 Bashkuev A, Se J, Aristov D, Ponomarenko A, Sinitshenkova N, Mahler H.
- 486 2012. Insects from the Buntsandstein of Lower Franconia and Thuringia.
- 487 *Paläontologische Zeitschrift* 86: 175–185.
- 488 Boulard M, Nel A. 1990. Sur deux cigales fossiles des terrains tertiaires de
- 489 la France (Homoptera, Cicadoidea). *Revue Francaise d'Entomologie* 12:
- 490 37–45.
- 491 Cohen KM, Finney SC, Gibbard PL, Fan J-X. 2013. The ICS International
- 492 Chronostratigraphic Chart. *Episodes* 36: 199-204.
- 493 Cooper KW. 1941. *Davispia bearcreekensis* Cooper, A new cicada from the
- 494 Paleocene, with a brief review of the fossil Cicadidae. *American Journal*
- 495 *of Science* 239: 286-304.
- 496 Drummond AJ, Suchard MA, Xie D, Rambaut A. 2012. Bayesian
- 497 phylogenetics with BEAUti and the BEAST 1.7. *Molecular Biology and*
- 498 *Evolution* 29: 1969–1973.
- 499 Flores RM, Bader LR. 1999. Fort Union coal in the Powder River Basin,
- 500 Wyoming and Montana: A synthesis. *U.S. Geological Survey Professional*
- 501 *Paper* 1625-A: 1-71.
- 502 Fujiyama I. 1969. A Miocene Cicada from Nasu, with an additional record of
- 503 a Pleistocene cicada from Shiobara, Japan. *Bulletin of the National*
- 504 *Science Museum, Tokyo*: 12: 863-875.
- 505 Fujiwara O, Yanagisawa Y, Irizuki T, Shimamoto M, Hayashi H. Danhara
- 506 T, Fuse K, Iwano H. 2008. Chronological data for the Middle Miocene to

- 507 Pliocene sequence around the southwestern Sendai Plain, with special
508 reference to the uplift history of the Ou Backbone Range. *Bulletin of*
509 *Geological Survey Japan* 59: 423–438.
- 510 Grande L. 1980. Paleontology of the Green River Formation with a review of
511 the fish fauna. *The Geological Survey of Wyoming Bulletin* 63: 1-333.
- 512 He HY, Wang XL, Jin F, Zhou ZH, Wang F, Yang LK, Ding X, Boven A, Zhu
513 RX. 2006. The $^{40}\text{Ar}/^{39}\text{Ar}$ dating of the early Jehol Biota from Fengning,
514 Hebei Province, northern China. *Geochemistry, Geophysics, Geosystems*
515 7: Q04001. doi:10.1029/2005GC001083 ISSN: 1525-2027
- 516 Hill KBR, Marshall DC, Moulds MS, & Simon C. 2015. Molecular
517 phylogenetics, diversification, and systematics of *Tibicen* Latreille, 1825
518 and allied cicadas of the tribe Cryptotympanini, with three new genera
519 and emphasis on species from the USA and Canada (Hemiptera:
520 Auchenorrhyncha: Cicadidae). *Zootaxa* 3985: 219–251.
- 521 Ho S. 2008. The molecular clock and estimating species divergence. *Nature*
522 *Education* 1: 168.
- 523 Ho SY, Phillips MJ, Cooper A, Drummond AJ. 2005. Time dependency of
524 molecular rate estimates and systematic overestimation of recent
525 divergence times. *Molecular Biology & Evolution* 2: 1561–1568.
- 526 Hong YC. 1982. Mesozoic Fossil Insects of Jiuquan Basin in Gansu
527 Province. Clapham. In Chinese.
- 528 Johnson KP *et al.* 2018. Phylogenomics and the evolution of hemipteroid
529 insects. *Proceedings of the National Academy of Sciences of the United*

- 530 *States of America*: 115: 12775-12780.
- 531 Kaulfuss U, Moulds M. 2015. A new genus and species of tettigarctid cicada
532 from the early Miocene of New Zealand: *Paratettigarcta zealandica*
533 (Hemiptera, Auchenorrhyncha, Tettigarctidae). *ZooKeys* 484: 83–94.
- 534 Liu Y, Qiu Y, Wang X, Yang H, Hayashi H, Wei C. 2018. Morphological
535 variation, genetic differentiation and phylogeography of the East Asia
536 cicada *Hyalessa maculaticollis* (Hemiptera: Cicadidae). *Systematic*
537 *Entomology* 43: 308–329.
- 538 Liu Y, Pham HT, He Z, Wei C. 2020. Phylogeography of the cicada
539 *Platypleura hilpa* in subtropical and tropical East Asia based on
540 mitochondrial and nuclear genes and microsatellite markers.
541 *International Journal of Biological Macromolecules* 151: 529-544.
- 542 Łukasik P, Nazario K, Van Leuven JT, Campbell MA, Meyer M, Michalik A,
543 Pessacq P, Simon C, Veloso C, McCutcheon JP. 2018. Multiple origins of
544 interdependent endosymbiotic complexes in a genus of cicadas.
545 *Proceedings of the National Academy of Sciences of the United States of*
546 *America* 115: E226–E235.
- 547 Liu Y, Qiu Y, Wang X, Yang H, Hayashi H, Wei C. 2018. Morphological
548 variation, genetic differentiation and phylogeography of the East Asia
549 cicada *Hyalessa maculaticollis* (Hemiptera: Cicadidae). *Systematic*
550 *Entomology* 43: 308–329.
- 551 Marshall DC. 2008. A simple method for bracketing absolute divergence
552 times on molecular phylogenies using multiple fossil calibration points.

- 553 American Naturalists 171: 726-42.
- 554 Marshall DC, Hill KBR, Moulds MS, Vanderpoo, D, Cooley JR, Mohagan
- 555 AB. Simon C. 2016. Inflation of molecular clock rates and dates:
- 556 Molecular phylogenetics, biogeography, and diversification of a global
- 557 cicada radiation from Australasia (Hemiptera: Cicadidae: Cicadettini).
- 558 *Systematic Biology* 65: 16–34.
- 559 Marshall DC, Moulds M, Hill KBR, Price BW, Wade EJ, Owen CL, Goemans
- 560 G, Marathe K, Sarkar V, Cooley JR, Sanborn AF, Kunte K, Villet MH,
- 561 Simon C. 2018. A molecular phylogeny of the cicadas (Hemiptera:
- 562 Cicadidae) with a review of tribe and 926 subfamily classification.
- 563 *Zootaxa* 4424: 1–64.
- 564 Misof B *et al.* 2014. Phylogenomics resolves the timing and pattern of insect
- 565 evolution. *Science* 346: 763-767.
- 566 McIntosh WC, Geissman JW, Chapin CE, Kunk MJ, Henry CD. 1992.
- 567 Calibration of the latest Eocene-Oligocene geomagnetic polarity time
- 568 scale using 40Ar/39Ar dated ignimbrites. *Geology* 20: 459-463.
- 569 Moulds MS. 2018. Cicada fossils (Cicadoidea: Tettigarctidae and Cicadidae)
- 570 with a review of the named fossilised Cicadidae. *Zootaxa* 4438: 443–470.
- 571 Osozawa S, Shinjo R, Armig R, Watanabe Y, Horiguchi T, Wakabayashi J.
- 572 2012. Palaeogeographic reconstruction of the 1.55 Ma synchronous
- 573 isolation of the Ryukyu Islands, Japan, and Taiwan and inflow of the
- 574 Kuroshio warm current. *International Geology Review* 54: 1369-1388.
- 575 Osozawa S, Shiyake S, Fukuda H, Wakabayashi J. 2017. Quaternary

- 576 vicariance of Platycleura (Cicadidae) in Japan, Ryukyu, and Taiwan
577 islands. *Biological Journal of the Linnean Society* 121: 185-199.
- 578 Papadopoulou A, Anastasiou I, Vogler AP. 2010. Revisiting the insect
579 mitochondrial molecular clock: The mid-Aegean trench calibration.
580 *Molecular Biology and Evolution* 27: 1659–1672.
- 581 Perrichot V, Nel A, Néraudeau D. 2005. Gerromorphan bugs in Early
582 Cretaceous French amber (Insecta: Heteroptera): first representatives of
583 Gerridae and their phylogenetic and palaeoecological implications.
584 *Cretaceous Research* 26: 793-800.
- 585 Petrusevičius JF. 2011. Paleogene insects from Maíz Gordo Formation,
586 northwest Argentina: Taphonomy, diversity and paleobiogeography, 337-
587 347. In Salfity JA, Marquillas RA, eds., *Cenozoic Geology of the Central*
588 *Andes and Argentina* SCS.
- 589 Piirainen M, Liebisch, O, Kadereit G. 2017. Phylogeny, biogeography,
590 systematics and taxonomy of Salicornioideae (Amaranthaceae /
591 Chenopodiaceae) – A cosmopolitan, highly specialized halophyte
592 lineage dating back to the Oligocene. *TAXON* 66: 109–132
- 593 Poinar Jr G, Gene Kritsky G. 2011. Morphological conservatism in the
594 foreleg structure of cicada hatchlings, *Burmacicada protera* n. gen., n. sp.
595 in Burmese amber, *Dominicicada youngi* n. gen., n. sp. in Dominican
596 amber and the extant *Magiccicada septendecim* (L.) (Hemiptera:
597 Cicadidae). *Historical Biology* 1: 1-6.
- 598 Popov YA. 1996. The first record of a fossil water bug from the Lower

599 Jurassic of Poland (Heteroptera: Nepomophrha: Belostomatidae). *Polskie*
600 *Pismo Entomologiczne* 65: 101-105.

601 Sage RF. 2004. The evolution of C4 photosynthesis. *New Phytologist* 161:
602 341–370.

603 Shi G, Grimaldi DA, Harlow GE, Wang J, Wang J, Yang M, Lei W, Li Q, Li
604 X. 2012. Age constraint on Burmese amber based on U-Pb dating of
605 zircons. *Cretaceous Research* 37: 155-163.

606 Simon C, Gordon ERL, Moulds MS, Cole JA, Haji D, Lemmon AR, Lemmon
607 EM, Kortyna M, Nazario K, Wade EJ, Meister RC, Goemans G, Chiswell
608 SM, Pessacq P, Veloso C, Mccutcheon JP, Łukasik P. 2019. Off-target
609 capture data, endosymbiont genes and morphology reveal a relict lineage
610 that is sister to all other singing cicadas. *Biological Journal of the*
611 *Linnean Society* 128: XX-YY.

612 Smith ME, Singer B, Carroll A. 2003. 40 Ar/39 Ar geochronology of the
613 Eocene Green River Formation, Wyoming. *Geological Society of America*
614 *Bulletin* 115: 549–565.

615 Suchard MA, Lemey P, Baele G, Ayres DL, Drummond AJ, Rambaut A.
616 2018. Bayesian phylogenetic and phylodynamic data integration using
617 BEAST 1.10. *Virus Evolution* 4: vey016.

618 Szwedo J, Nel A. 2011. The oldest Aphid insect from the Middle Triassic of
619 the Vosges, France. *Acta Palaeontologica Polonica* 56: 757-766.

620 Taira A. 2007. Search the Earth History, Geology 3, pp. 396. Iwanami
621 Shoten, Publishers, Tokyo. (in Japanese)

- Walther H, Kvacek Z. 2007. Early Oligocene flora of Seifhennersdorf (Saxony). *Acta Musei Nationalis Pragae, Series B, Historia Naturalis* 63: 85–174.
 - Whalley PES. 1985. The systematics and palaeogeography of the Lower Jurassic insects of Dorset, England. *Bulletin of the British Museum of Natural History (Geology)* 39: 107-189.
 - Woodburne MO, Goin FJ, Raigemborn MS, Heizler M, Gelfo JN, Oliveira EV. 2014. Revised timing of the South American early Paleogene land mammal ages. *Journal of South American Earth Sciences* 54: 109–119.
 - Yoshikawa T. 2005. Paleoenvironmental change and age of the Miocene Kanomatazawa Formation in the Shiobara area, Tochigi Prefecture, central Japan. *Journal of Geological Society of Japan* 111: 39-49. In Japanese with English abstract.
 - Zhang H. 1997. Early Cretaceous insects from the Dalazi Formation of the Zhixin Basin, Jilin Province, China. *Palaeoworld* 7: 75-103.
 - Zucol AF, Krause JM, Brea M, Raigemborn MS, Matheos SD. 2018. Emergence of grassy habitats during the greenhouse–icehouse systems transition in the Middle Eocene of southern South America. *Ameghiniana* 55: 451–482.
- Fig. 1.** Simplified cicada timetree built by BEAST v1.X, applying 1,534 bp COI sequence. Inserted figure: Base substitution rate (= rate median

shown at each node; mutations per bp per million years) vs age (= posterior age shown at each node) diagram. Purple curve with equation: Exponential trendline calculated using Excel.

Fig. 2. Cicada timetree built by BEAST v1.X, applying 1,534 bp COI sequence. OUTs with isolate number: our own analyzed specimens shown in Table 1, and others: from GenBank / DDJB. In outgroup Hemiptera, #: analyzed family by Johnson et al. (2018), % analyzed family by Misof et al. (2014). Note that the outgroup is calibrated by the single point point A1. Inserted figure: Base substitution rate (= rate median shown at each node; mutations per bp per million years) vs age (= posterior age shown at each node) diagram. Purple curve with equation: Exponential trendline calculated using Excel.

Fig. 3. Cicada timetree built by BEAST v1.X, applying 1,534 bp COI and 874 bp 18S rRNA sequences. OUTs with isolate number: our own analyzed specimens shown in Table 1, and others: from GenBank / DDJB. In outgroup Hemiptera, #: analyzed family by Johnson et al. (2018), % analyzed family by Misof et al. (2014). Inserted figure: Base substitution rate (= rate median shown at each node; mutations per bp per million years) vs age (= posterior age shown at each node) diagram. Purple curve with equation: Exponential trendline calculated using Excel.

668 **Table 1.** East Asian cicada species collected and analyzed in this paper.

Fig.3

isolate	country	species	accession no. COI	18S rRNA	collection date	collected by
pp44a	South Korea: Busan	<i>Platypleura kaempferi</i> (Fabricius, 1794)	AB897523 LC086120	LC086101	2011.7.13	Soichi Osozawa
pp53	Japan: Honshu, Tobi-shima	<i>Platypleura kaempferi</i> (Fabricius, 1794)	LC086279 LC086066	LC086137	2014.7.31	Soichi Osozawa
pp54	China: Zhejiang	<i>Platypleura kaempferi</i> (Fabricius, 1794)	LC086280 LC086067	LC086138	2014.8.13	Akira Mashima
pp26a	Taiwan: Yangmingshan park	<i>Platypleura kaempferi</i> (Fabricius, 1794)	AB897530 LC086092	LC086163	2013.6.7	Soichi Osozawa
pp6R	Japan: Ryukyu, Ishigaki-jima	<i>Platypleura yayeyamana</i> Matsumura, 1917	AB897534 LC086072	LC086143	2010.7.5	Soichi Osozawa
pp21	Japan: Ryukyu, Ishigaki-jima	<i>Platypleura yayeyamana</i> Matsumura, 1917	AB897535 LC086085	LC086156	2010.7.5	Soichi Osozawa
pp27	Japan: Ryukyu, Iriomote-jima	<i>Platypleura yayeyamana</i> Matsumura, 1917	AB897540 LC086096	LC086167	2013.6.14	Soichi Osozawa
pp9R	Japan: Ryukyu, Miyako-jima	<i>Platypleura miyakona</i> (Matsumura, 1917)	AB897557 LC086073	LC086144	2011.7.3	Soichi Osozawa
pp3	Taiwan: Taipei, Entsu-ji	<i>Platypleura takasagana</i> Matsumura, 1917	AB897556 LC086071	LC086142	2012.5.27	Shusuke Osozawa
pp40a	Japan: Ryukyu, Amami-Oshima	<i>Platypleura kaempferi</i> Matsumura, 1917	AB897542 LC086115	LC086186	2013.7.7	Soichi Osozawa
pp15d	Japan: Ryukyu, Tokuno-shima	<i>Platypleura kaempferi</i> Matsumura, 1917	AB897548 LC086083	LC086154	2012.7.2	Soichi Osozawa
pp32	Japan: Ryukyu, Okinawa-jima	<i>Platypleura kaempferi</i> Matsumura, 1917	AB897561 LC086103	LC086174	2013.6.20	Soichi Osozawa
pp10b	Japan: Ryukyu, Kume-jima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897563 LC086075	LC086148	2012.6.23	Soichi Osozawa
pp13b	Japan: Ryukyu, Okinawa-jima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897562 LC086079	LC086150	2010.6.22	Soichi Osozawa
pp15d	Japan: Ryukyu, Tokuno-shima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897565 LC086080	LC086151	2012.7.2	Soichi Osozawa
pp23	Japan: Ryukyu, Amami-Oshima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897566 LC086087	LC086158	2010.6.23	Soichi Osozawa
pp35a	Japan: Ryukyu, Okinoerabu-jima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897569 LC086108	LC086179	2013.6.28	Soichi Osozawa
pp42	Japan: Ryukyu, Amami-Oshima	<i>Platypleura kuroiwa</i> Matsumura, 1917	AB897567 LC086117	LC086188	2013.7.9	Soichi Osozawa
pp20	Taiwan: Chiang Rai	<i>Platypleura nobilis</i> (Germar, 1830)	AB897581 LC086133	LC086204	2008.8.5	Tetsuo Miyashita
pp1	Philippine: Babuyan island	<i>Platypleura hilpa</i> Walker, 1850	AB897582 LC086134	LC086205	2011.7.5	Kei Nishiguro
pp58a	Philippine: Luzon island, Mt. Mayon	<i>Platypleura hilpa</i> Walker, 1850	(AB897582) LC086135	LC086206	2014.4.5	Kei Nishiguro
kum25	Japan: Ryukyu, Amami-Oshima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466803	LC466820	2018.7.5	Kenichi Kanai
kum27A	Japan: Ryukyu, Amami-Oshima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466804	LC466821	2018.7.6	Kenichi Kanai
kum4	Japan: Ryukyu, Kume-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466805	LC466822	2014.7.5	Fumiyasu Sato
kum28A	Japan: Ryukyu, Okinawa-jima, Minna-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466806	LC466823	2018.6.28	Satoru Nitta
kum15	Japan: Ryukyu, Miyako-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466807	LC466824	2011.7.4	Soichi Osozawa
kum9	Japan: Ryukyu, Tarama-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466808	LC466825	2016.6.14	Soichi Osozawa
kum19	Japan: Ryukyu, Ishigaki-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466809	LC466826	2017.6.26	Hiroshi Irino
kum33	Japan: Ryukyu, Kuro-shima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466810	LC466827	2018.7.18	Soichi Osozawa
kum36A	Japan: Ryukyu, Iriomote-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466811	LC466828	2018.9.7	Hiroshi Irino
kum18	Japan: Ryukyu, Yonaguni-jima	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466812	LC466829	2019.7.10	Minoru Saijo
kum21	Japan: Honshu, Itami	<i>Cryptotympana facialis</i> (Walker, 1858)	LC466813	LC466830	2018.7.21	Soichi Osozawa
kum34	Taiwan: Wulai	<i>Cryptotympana holsti</i> Distant, 1904	LC466814	LC466831	2018.7.4	Soichi Osozawa
kum1	China: HongKong	<i>Cryptotympana mandarina</i> Distant, 1891	LC466815	LC466832	2013.5.29	Soichi Osozawa
kum12	Taiwan: Taipei Zoo	<i>Cryptotympana takasagana</i> Kato, 1925	LC466816	LC466833	2016.8.28	Soichi Osozawa
abr5	Japan: Honshu, Ito	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508809	LC508884	2013.7.12	Soichi Osozawa
N-ec1	Japan: Ryukyu, Minami Daito-jima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508810	LC508885	2013.8.13	Akira Mashima
S-ec2	Taiwan: Taichung	<i>Euterpnosia olivacea</i> Kato, 1927	LC508811	LC508886	2013.7.30	Soichi Osozawa
S-ec3	Japan: Ryukyu, Yonaguni-jima	<i>Euterpnosia iwazaki</i> (Matsumura, 1913)	LC508812	LC508887	2012.4.5	Ryosuke Sadaki
S-ec4a	Japan: Ryukyu, Ishigaki-jima	<i>Euterpnosia iwazaki</i> (Matsumura, 1913)	LC508813	LC508888	2010.8.8	Soichi Osozawa
N-ec5a	Japan: Ryukyu, Kume-jima	<i>Euterpnosia chibensis okinawana</i> Ishihara, 1968	LC508814	LC508889	2011.6.29	Soichi Osozawa
N-ec6a	Japan: Ryukyu, Okinawa-jima	<i>Euterpnosia chibensis okinawana</i> Ishihara, 1968	LC508815	LC508890	2011.6.25	Soichi Osozawa
N-ec6a-2	Japan: Ryukyu, Okinawa-jima	<i>Euterpnosia chibensis okinawana</i> Ishihara, 1968	LC508816	LC508891	2012.6.23	Soichi Osozawa
N-ec7a	Japan: Ryukyu, Tokuno-shima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508817	LC508892	2010.6.25	Soichi Osozawa
N-ec7b	Japan: Ryukyu, Tokuno-shima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508818	LC508893	2012.7.2	Soichi Osozawa
N-ec8a	Japan: Ryukyu, Amami Oshima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508819	LC508894	2010.6.28	Soichi Osozawa
N-ec9	Japan: Honshu, Kochi	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508820	LC508895	2011.8.1	Soichi Osozawa
S-ec10	Taiwan: Hualien	<i>Euterpnosia viridifrons</i> Matsumura, 1917	LC508821	LC508896	2013.6.4	Soichi Osozawa
S-ec12a	Taiwan: Yangmingshan	<i>Euterpnosia gina</i> Kato, 1931	LC508822	LC508897	2013.6.7	Soichi Osozawa
S-ec13	Japan: Ryukyu, Ishigaki-jima	<i>Euterpnosia iwazaki</i> (Matsumura, 1913)	LC508823	LC508898	2013.6.15	Soichi Osozawa
N-ec14-1	Japan: Ryukyu, Itoya-jima	<i>Euterpnosia chibensis okinawana</i> Ishihara, 1968	LC508824	LC508899	2013.6.21	Soichi Osozawa
N-ec15	Japan: Ryukyu, Okinoerabu-jima	<i>Euterpnosia chibensis okinawana</i> Ishihara, 1968	LC508825	LC508900	2013.6.28	Soichi Osozawa
N-ec17-1	Japan: Ryukyu, Amami Oshima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508826	LC508901	2013.7.9	Soichi Osozawa
N-ec18a	Japan: Kyushu, Kagoshima	<i>Euterpnosia chibensis</i> Matsumura, 1917	LC508827	LC508902	2013.8.3	Heruhiko Fukuda et al
ec19	China: Anhui	<i>Euterpnosia</i> sp.	LC508828	LC508903	2013.6.10	Akira Mashima
min-mn2	Japan: Ryukyu, Yonaguni-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508829	LC508904	2011.6.29	Soichi Osozawa
min-mn3	Japan: Ryukyu, Ishigaki-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508830	LC508905	2010.5.1	Soichi Osozawa
min-mn4	Japan: Ryukyu, N Iriomote-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508831	LC508906	2010.4.30	Soichi Osozawa
min-mn5	Japan: Ryukyu, Miyako-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508832	LC508907	2011.4.25	Soichi Osozawa
min-mn6	Japan: Ryukyu, Okinawa-jima, Tsuken-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508833	LC508908	2012.6.6	Satoru Nitta
min-mn9a	Japan: Ryukyu, Okinawa-jima, Yaguchi-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508834	LC508909	2014.5.11	Atsuko Nitta
min-mn10a	Japan: Ryukyu, Okinawa-jima, Tamagusuku	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508835	LC508910	2014.5.30	Yusui Watanabe
min-mn12a	Japan: Ryukyu, Kohama-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508836	LC508911	2014.7.6	Soichi Osozawa
min-mn14a	Japan: Ryukyu, S Iriomote-jima	<i>Mogannia minuta</i> (Matsumura, 1907)	LC508837	LC508912	2016.6.18	Soichi Osozawa
mn18	Taiwan: Hualien	<i>Mogannia formosana</i> Matsumura, 1907	LC508838	LC508913	2013.6.2	Soichi Osozawa
heb-mn2B	Taiwan: Hualien	<i>Mogannia hebes</i> Walker, 1858	LC508839	LC508914	2013.6.2	Soichi Osozawa
heb-mn3B	Taiwan: Hualien	<i>Mogannia hebes</i> Walker, 1858	LC508840	LC508915	2013.6.3	Soichi Osozawa
heb-mn5B	Taiwan: Yangmingshan	<i>Mogannia hebes</i> Walker, 1858	LC508841	LC508916	2013.6.7	Soichi Osozawa
heb-mn6B	Taiwan: Yangmei	<i>Mogannia hebes</i> Walker, 1858	LC508842	LC508917	2013.6.8	Soichi Osozawa
heb-mn7aB	China: HongKong	<i>Mogannia hebes</i> Walker, 1858	LC508843	LC508918	2013.5.30	Akira Mashima
heb-mn13aB	China: Hubei	<i>Mogannia hebes</i> Walker, 1858	LC508844	LC508919	2014.6.18	Soichi Osozawa
tuk1	Japan: Kyushu, Tsushima	<i>Meimuna opalifera</i> (Walker, 1850)	LC508845	LC508920	2013.7.18	Soichi Osozawa
tuk2	Japan: Honshu, Okino-shima	<i>Meimuna opalifera</i> (Walker, 1850)	LC508846	LC508921	2013.8.28	Soichi Osozawa
tuk3a	Japan: Izu, Hachijo-jima	<i>Meimuna opalifera</i> (Walker, 1850)	LC508847	LC508922	2014.7.16	Soichi Osozawa
tuk4	Japan: Tokara, Nakano-shima	<i>Meimuna opalifera</i> (Walker, 1850)	LC508848	LC508923	2010.8.31	Soichi Osozawa
tuk5	Japan: Honshu, Sendai	<i>Meimuna opalifera</i> (Walker, 1850)	LC508849	LC508924	2014.9.6	Soichi Osozawa
tuk6	China: Zhejiang	<i>Meimuna opalifera</i> (Walker, 1850)	LC508850	LC508925	2014.8.13	Soichi Osozawa
tuk10	Japan: Kyushu, Yaku-shima	<i>Meimuna opalifera</i> (Walker, 1850)	LC508851	LC508926	2017.10.11	Haruo Fukuda
tuk12	Taiwan: Taipei	<i>Meimuna opalifera</i> (Walker, 1850)	LC508852	LC508927	2017.11.6	Soichi Osozawa
ohs1	Japan: Ryukyu, Okinawa-jima	<i>Meimuna oshimensis</i> (Matsumura, 1905)	LC508853	LC508928	2010.6.23	Soichi Osozawa
ohs2	Japan: Ryukyu, Amami Oshima	<i>Meimuna oshimensis</i> (Matsumura, 1905)	LC508854	LC508929	2010.9.9	Soichi Osozawa
ohs3a	Japan: Ryukyu, Ishigaki-jima	<i>Meimuna iwazaki</i> (Matsumura, 1913)	LC508855	LC508930	2014.9.5	Tadafumi Nakada
ohs4a	Japan: Ryukyu, Kume-jima	<i>Meimuna oshimensis</i> (Matsumura, 1905)	LC508856	LC508931	2014.8.15	Soichi Osozawa
ohs7a	Japan: Ryukyu, Itoya-jima	<i>Meimuna oshimensis</i> (Matsumura, 1905)	LC508857	LC508932	2015.9.18	Soichi Osozawa
kur1	Japan: Ryukyu, Amami Oshima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508858	LC508933	2010.9.9	Soichi Osozawa
kur2	Japan: Tokara, Takara-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508859	LC508934	2010.9.3	Soichi Osozawa
kur3a	Japan: Tokara, Nakano-shima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508860	LC508935	2010.8.29	Soichi Osozawa
kur4a	Japan: Ryukyu, Kume-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508861	LC508936	2014.8.15	Soichi Osozawa
kur5	Japan: Ryukyu, Okinawa-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508862	LC508937	2015.9.17	Soichi Osozawa
kur6	Japan: Ryukyu, Okinawa-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508863	LC508938	2015.9.17	Soichi Osozawa
kur7a	Japan: Ryukyu, Itoya-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508864	LC508939	2015.9.18	Soichi Osozawa
kur8	Japan: Kyushu, Kagoshima, Cape Sata	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508865	LC508940	2017.10.4	Nobuharu Kumagai
kur9	Japan: Kyushu, Yaku-shima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508866	LC508941	2017.10.10	Haruo Fukuda
kur10	Japan: Ryukyu, Kikai-jima	<i>Meimuna kuroiwa</i> (Matsumura, 1917)	LC508867	LC508942	2017.11.10	Nobuharu Kumagai
TH-pomp1a	Taiwan: Hualien	<i>Pomponia linearis</i> Walker, 1850	LC508868	LC508943	2013.6.4	Soichi Osozawa
TH-pomp11	Japan: Ryukyu, Ishigaki-jima	<i>Pomponia linearis</i> Walker, 1850	LC508869	LC508944	2014.8.21	Tadafumi Nakada
TH-pomp9a	China: Zhejiang	<i>Pomponia linearis</i> Walker, 1850	LC508870	LC508945	2014.7.25	Akira Mashima
pomp3	Japan: Ryukyu, Amami Oshima	<i>Tanna japonensis</i> Distant, 1892	LC508871	LC508946	2013.7.7	Soichi Osozawa
pomp5	Japan: Honshu, Sendai	<i>Tanna japonensis</i> Distant, 1892	LC508872	LC508947	2014.7.21	Soichi Osozawa
pomp7b	China: Zhejiang	<i>Tanna</i> sp.	LC508873	LC508948	2014.8.13	Akira Mashima
pomp10	Japan: Ryukyu, Ishigaki-jima	<i>Tanna japonensis ishigakiana</i> (Kato, 1960)	LC508874	LC508949	2014.8.21	Tadafumi Nakada
pomp2a	Taiwan: Yangmingshan	<i>Tanna sozanensis</i> Kato, 1926	LC508875	LC508950	2013.6.7	Soichi Osozawa
abr1	Japan: Ryukyu, Itoya-jima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508876	LC508951	2013.6.21	Soichi Osozawa
abr2	Japan: Ryukyu, Okinawa-jima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508877	LC508952	2010.6.22	Soichi Osozawa
abr3f	Japan: Ryukyu, Tokashiki-jima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508878	LC508953	2013.6.24	Soichi Osozawa
abr11	Japan: Ryukyu, Kume-jima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508879	LC508954	2014.6.22	Fumiyasu Sato
abr3	Japan: Ryukyu, Tokuno-shima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508880	LC508955	2013.7.5	Soichi Osozawa
abr4	Japan: Ryukyu, Amami-Oshima	<i>Graptopsaltria bimaculata</i> Kato, 1925	LC508881	LC508956	2013.7.8	Soichi Osozawa
abr6	Japan: Hokkaido	<i>Graptopsaltria nigrofusca</i> (Motschulsky, 1866)	LC508882	LC508957	2013.8.16	Shusuke Osozawa
minin-abr7	Japan: Hokkaido	<i>Hyalessa maculicollis</i> (Motschulsky, 1866)	LC508883	LC508958	2013.8.16	Shusuke Osozawa
minin-abr9	Japan: Honshu, Okino-shima	<i>Hyalessa maculicollis</i> (Motschulsky, 1866)	LC508884	LC508959	2013.8.28	Soichi Osozawa

0000001