Reconstruction of an extinct soundscape reveals ultrasoniccommunication in the Jurassic

Authors: Jun-Jie Gu, Fernando Montealegre-Z, Thorin Jonsson, Charlie Woodrow, Emine Celiker, Md Niamul Islam, Jackson B. Linde, Fabio Sarria-S, Fuming Shi, Hojun Song, Daniel Robert &, Dong Ren

Supplementary methods

Phylogenomic analysis

Data processing, filtering, and phylogenomic analysis

Adapter contamination and low-quality bases were removed using Trimmomatic[85], and trimmed reads were assembled de novo using SOAPdenovo2[86]. To assign assembled sequences to orthologous loci with high specificity, we used Orthograph with an Orthoptera-focused ortholog database. Orthograph[87] was used to screen assemblies and identify putative orthologs, thereby minimizing inclusion of paralogs and non-target contaminants while standardizing locus identities across samples. Thousands of recovered loci are generated at this step.

Ortholog alignment construction and orthology quality control

Orthologous sequences were subsequently processed through the 1KITE cleaning pipeline to further curate orthology and standardize locus sets[88]. This step included additional orthology checking and cleaning procedures, followed by multiple sequence alignment of each orthologous locus using MAFFT (v7.130b)[89]. The resulting per-locus alignments formed the initial matrix for downstream filtering and phylogenetic inference.

Site-level filtering and sequence-level cleanup

We filtered alignments a custom python script (01_filter_gap_alignments.py) allowing up to 90% gaps per column of the alignment, and up 35% missing data (within a solid empirical threshold)6 per row with a minimum of 100bp and at least 4 taxa present - this was done as utilizing small alignments and limited filtering allows for true signal to be retained[90]. After filtering, to deal with initial transcriptomic biases (these data consist of thousands more genes than typical hybrid capture, and can thus overwhelm the lesser amount of data and transcriptomic data can be drawn together artificially) we used our custom transcript_only_filter.py to remove alignments consisting of only transcriptomic sequences.

Gene tree inference and rogue taxon mitigation

To assess gene-level topological variation and identify problematic taxa, we inferred a maximum likelihood gene tree for each filtered locus alignment in RAxML-NG v1.2.0[91]. These were run with the GTR+G model and 100 bootstrap trees. These per-locus trees were used to examine patterns of concordance and conflict among loci and to identify sequences exhibiting anomalous placement likely driven by missing data, alignment artifacts, or contamination. We then applied TreeShrink v1.3.9[92] to the gene tree set to detect and mitigate the impact of rogue taxa (i.e., taxa producing unusually long branches or unstable placements across loci). Taxa flagged as likely to distort inference were removed or down-weighted according to the TreeShrink workflow[92], thereby improving robustness of downstream concatenated and time-calibrated analyses. After TreeShrink, we took updated alignments (adjusted by the program) and re-estimated gene trees with rogue taxa removed.

Monophyly screening to prevent technology-driven clustering

Because our dataset combines hybrid capture and transcriptome-derived sequences, we implemented an additional safeguard to ensure that sequences were not clustering artificially by data type rather than evolutionary history. We used a custom monophyly filter (final_monophyly_checker.py) to test whether transcriptome-derived sequences formed spurious monophyletic groups across loci or in the concatenated topology. Loci or taxa showing evidence of technology-associated, artificial monophyletic grouping by data type were flagged and removed prior to final inference, similarly to Frandsen et al.[93].

Concatenated phylogenomic inference and partition optimization

We concatenated the final 1,077 locus alignments into a supermatrix using AMAS.py script[94] and generated an initial partitioning scheme with one partition per orthologous locus. We inferred the maximum likelihood phylogeny of the 625,593 bp supermatrix in IQ-TREE v3.0.1[95] using comprehensive model and partition optimization. Specifically, we used ModelFinder[96] with partition merging enabled (MFP + MERGE) and the greedy algorithm (rcluster) with parameter 10, allowing the data to determine an optimised, reduced partition scheme[97]. Node support was assessed with 1,000 ultrafast bootstrap replicates[98] with the "-bnni" flag added to reduce the impact of known ultrafast bootstrap inflation toward values of 100 and 1,000 SH-aLRT replicates, providing complementary measures of branch reliability.

Acoustic and wing motion recordings

Stridulatory wing movements and the associated sound production were recorded from *Cyphoderris monstrosa* (3 males), *Tarragoilus diuturnus* (2 males), *Gryllus bimaculatus* (9 males), and, and *Panacanthus pallicornis* (8 males) using protocols well established by the authors. Acoustic signals were captured using a calibrated 1/8″ microphone (Brüel & Kjær Type 4138 connected to a preamplifier Type 2633) and amplified with a B&K1708 conditioning amplifier (Brüel & Kjaer, Nærum, Denmark). Wing movements were monitored using an opto-electronic device[99,100]. To facilitate recordings from dorsal or posterior views, a piece of reflective tape (Scotchlite 7610 and 8850, 3M) was affixed to the left tegmen. Sound and wing movement signals were recorded on separate channels using PSV acquisition software (Polytec GmbH, Waldbronn, Germany), which employed a National Instruments (NI) acquisition board. A high-pass filter was set at 2 kHz for the sound recordings, a low-pass filter with a cut-off at 5 kHz for the wing motion, and a sampling frequency of 512 kHz. Experimental conditions were maintained at a temperature of 24-26 °C and a relative humidity of 35–40%.

Laser Doppler Vibrometry

Wing resonance was measured in the same species above [*Cyphoderris monstrosa* (3 males), *Tarragoilus diuturnus* (8 males), *Gryllus bimaculatus* (47 males), and, and *Panacanthus pallicornis* (8 males)], using micro-scanning laser Doppler vibrometry (LDV; PSV-500, Polytec GmbH, Waldbronn, Germany). In these experiments, the insect was mounted on a horizontal brass platform following the methodology outlined by Montealegre-Z et al.[100]. The dorsal region of the wings was stimulated using synthetic sound and scanned with the LDV across a grid of approximately 1,000 points, utilising a sample rate of 128 kHz and averaging three measurements per point. The acoustic stimulation comprised broadband periodic chirps within the frequency range of 1.5-50 kHz. The sound pressure of the stimulus was calibrated to ensure a consistent sound pressure level (SPL) of 60 ± 1.5 dB (re. 20) throughout the experimental range. Acoustic signals were generated by the internal data acquisition board of the PSV (PCI-4451; National Instruments, Austin, TX, USA), amplified (A-400, Pioneer, Kawasaki, Japan), and transmitted to a loudspeaker (Ultrasonic Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke, Germany) located 30 cm from the specimen. The reference signal was recorded with a 1/8″ condenser microphone placed horizontally near the wings (Brüel & Kjaer, 4138-A-015, and preamplifier model 2670, Brüel & Kjaer, Nærum, Denmark).

Micro-CT scanning of the hearing system in living forms

Specimens were scanned using a SkyScan 1172 µ-CT scanner (12.9 µm voxel size, 55 kV source voltage, 200 µA source current, 200 ms exposure, 0.2◦ rotation step; Bruker Corporation, Billerica, MA, USA). µ-CT projection images were reconstructed to produce orthogonal slices with NRecon (v.1.6.9.18, Bruker Corporation, Billerica, MA, USA). For 3D segmentation of tracheae, the orthogonal slice data were imported into Amira-Aviso 6.7 (Thermo Fisher Scientific, Waltham, Massachusetts, USA), and the lumen of the tracheae was selected using the magic wand tool every 5 slices throughout the whole dataset, followed by interpolation to connect slices. This selection was then used to generate a 3D surface and 2D image. The 3D image of the inner ear of the katydid (*Mimetica* sp.) is modified from Celiker et al[101].

Gaussian process regression for predicting syllable rates in fossilised insects

This study employed a machine learning approach to estimate the syllable rates of calling song of extinct orthopteran insects based on preserved the morphological and the simulated acoustic traits. Gaussian Process Regression (GPR) was selected as the core modelling technique due to its non-parametric, probabilistic framework, as it does not impose a fixed functional form on the data. GPR is well-suited to small-to-medium-sized biological datasets, making it appropriate for the limited number of extant species available for training[102]. Additionally, it enables the generation of confidence intervals alongside point predictions, allowing quantification of uncertainty, an important requirement when extrapolating to fossil taxa. The ability of the model to capture non-linear relationships while extrapolating to regions beyond the training data make GPR particularly effective for reconstructing extinct calling song.

Training Dataset and Features

The training dataset was compiled on four input features based on the morphological and the acoustic traits of extant and extinct species:

1. The stridulatory file length (mm)
2. Total number of teeth on the stridulatory file
3. The dominant frequency (kHz) of the species' acoustic signa
4. The syllable duration (ms) in the call.

The target variable was the syllable rate, calculated from the equation of 'syllable/wingstroke/phanotome rate changes with temperature' from existing literatures

(Supplementary Table 4)[103–111]. An ambient temperature of 20 °C was selected for the syllable-rate calculations as it sits near the centre of Jurassic climate estimates from multiple lines of evidence. Global multi-proxy compilations place mean surface values between ~19.6 °C (Early-Jurassic cool interval) and ~24.6 °C (Toarcian warm interval)[112]; high-latitude TEX-86 records from the Falkland Plateau yield consistently warm sea-surface temperatures of 26–30 °C during the Middle-to-Late Jurassic[113]; and a mid-latitude continental reconstruction for the Callovian Yanliao Biota indicates mean annual air temperatures of 15–20 °C[114]. A value of 20 °C therefore provides a representative midpoint for the range of Jurassic ambient conditions. Some species exhibited two distinct syllable rate–temperature equations, from which the higher syllable rate was selected for modelling to ensure a consistent and conservative estimate of the species' maximum acoustic performance. This approach establishes an upper bound for communication rate based on available data and avoids underestimating the insect's potential signalling capacity. Species with complex phanotomes (calling song consisting of multiple syllable and different syllable rate equation for different syllables) were ignored to reduce complexity of the model for prediction.

Kernel Construction

The GPR model's behaviour is governed by its kernel (covariance function), which defines similarity between data points. A composite kernel was constructed:

$$k(x, x') = c_1 \cdot Matern_v(\ell) + c_2 \cdot DotProduct + WhiteKernel$$

- The Matérn kernel $Matern_v(\ell)$ was used for modelling spatial smoothness, where ℓ is the characteristic length scale and $v \in \{0.5, 1.5, 2.5\}$ controls smoothness (from rough to smooth functions). The scaling constant c_1 modulates the variance of the function prior, essentially controlling how much influence the Matérn component has on the output. Larger values of c_1 allow the model to capture higher variability driven by smooth patterns in the input space. This kernel was chosen to capture local non-linearities while providing a tuneable smoothness prior, which has been effective in ecological and evolutionary models.

- The DotProduct kernel, scaled by c_2, captures linear relationships and supports extrapolation, which is critical when making predictions for fossil data lying outside the range of the extant species. The scaling constant c_2 defines how much weight this linear trend contributes to the overall model.

- A WhiteKernel captures small observational noise, allowing for non-zero variance even when inputs are identical and avoid overfitting.

Together, c_1 and c_2 balance the contributions of the Matérn and linear components of the model. This hybrid structure enhances the model's flexibility as it can fit nonlinear trends within the range of known species, while still being capable of generalising linearly the unseen, extrapolated fossil inputs.

Log-Transformation of the Target

Since syllable rate values must be positive and spanned an order of magnitude, the target syllable rate was transformed as:

$$y_{log} = log\ (y)$$

This transformation improves normality, variance stability, and ensures that predicted rates remain positive. After prediction, values were exponentiated:

$$\hat{y} = exp\ (\mu \pm \sigma)$$

where μ is the mean predicted log-rate and σ is the standard deviation from the GPR posterior.

Hyperparameter Tuning via Bayesian Optimisation

Kernel hyperparameters, including $\ell, \nu, \sigma_0, c_1, c_2$ and noise level were optimised using Bayesian optimisation via the Optuna framework to efficiently explores complex, non-convex hyperparameter spaces[115]. The objective function was defined as the negative mean squared error (MSE) over 3-fold cross-validation:

$$MSE = \frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2$$

The search for the parameters was terminated after 1000 trials or earlier if the best value did not improve for 200 consecutive iterations.

Model Evaluation

After training, model performance was evaluated on the full dataset using three metrics:

- Mean Absolute Error (MAE):

$$MAE = \frac{1}{n}\sum_{i=1}^{n}|y_i - \hat{y}_i|$$

Measures average absolute deviation, reflecting typical error magnitude.

- Root Mean Squared Error (RMSE):

$$RMSE = \sqrt{\frac{1}{n}\sum_{i=1}^{n}(y_i - \hat{y}_i)^2}$$

Emphasises larger errors due to squaring, useful for detecting outlier influence.

- Coefficient of Determination (R^2):

$$R^2 = 1 - \frac{\sum(y_i - \hat{y}_i)^2}{\sum(y_i - \bar{y})^2}$$

Represents the proportion of variance explained by the model. Values near 1 indicate high predictive accuracy.

All metrics were additionally normalised by the mean syllable rate to express relative performance.

Optimal hyper parameter

Following Bayesian optimisation, the Gaussian Process Regression model converged on the following kernel hyperparameters as optimal for predicting log-transformed syllable rates: $c_1 = 1.23$, $\ell = 1.46$, $\nu = 1.5$, $c_2 = 0.731$, $\sigma_0 = 0.136$, and $noise\ level = 5.95 \times 10^{-5}$. These values were used to construct the final kernel and train the model on the complete dataset.

Application to Fossil Data

The validated GPR model was applied to predict syllable rates in the fossil taxa. Each fossil species was represented by a vector of four inputs: stridulatory file length, tooth count, estimated calling frequency, and the estimated syllable duration. GPR outputs were converted from log space and plotted with standard deviation error bars to reflect prediction uncertainty.

For each fossil, the maximum possible rate was calculated to compare predicted values with the theoretical bound to ensure biological plausibility:

$$Max\ syllable\ rate = \frac{1000}{Syllable\ duration\ (ms)}$$

Model limitation

While GPR provides a powerful probabilistic framework for estimating syllable rates in extinct taxa, it is important to acknowledge the model's limitations in reconstructing full acoustic behaviours. The current model predicts syllable rate based on morphological and acoustic data under the assumption that these traits directly and consistently determine rhythmic sound production across taxa. However, this prediction represents only a single component of a more complex acoustic signal.

In particular, the model assumes that a species' call consists of simply regular, trill-like syllable production patterns. Real insect songs sometimes also include structured elements such as chirps, phrases, pauses, and modulations, shaped by neurophysiological controls, environmental pressures, and species-specific behavioural contexts which are not captured by morphological features alone. Finally, some fossils may fall outside the range of known species used to train the model and should be interpreted with caution.

Still, the model's predicted syllable rates serve as a biomechanical estimate of sound rhythm potential, useful primarily for reconstructing hypothetical trilling signals under idealised conditions rather than complete reconstructions of fossil sound.

Results – GPR model performance and evaluation

The trained Gaussian Process Regression (GPR) model demonstrated strong predictive performance when evaluated on the full dataset of extant insect species. The model was assessed using three standard regression metrics: mean absolute error (MAE), root mean squared error (RMSE), and the coefficient of determination (R^2). Predictions were made on the log-transformed syllable rate, then exponentiated to yield values in the original scale (syllables/second).

The mean absolute error (MAE) was 2.42 syllables/sec, corresponding to approximately 5.5% of the mean syllable rate in the dataset. This indicates that, on average, the model's predictions deviate from the true syllable rate by just over 2 syllables per second—a small discrepancy considering that some species reach rates over 90 syllables/sec. The root mean squared error (RMSE) was 4.10 syllables/sec (9.4%), showing that the model maintains good robustness even

when occasional larger errors occur, and that extreme outliers are not a concern. The model achieved an R^2 value of 0.966, indicating that 96.6% of the variance in the observed syllable rates is explained by the GPR model. This level of explanatory power is considered excellent in biological data modelling and suggests that the chosen features—file length, tooth count, carrier frequency, and syllable duration—collectively contain strong predictive signals for acoustic output.

Visual inspection of the actual vs predicted plot (see Extended Data, Fig 8b) confirms that majority of predicted values fall close to the identity line (y = x), with no systematic over- or under-prediction across the range. Slight underestimation is observed for some of the highest syllable rates, which likely reflects the relatively lower representation of high-rate species in the training data and the conservative nature of GPR models when extrapolating near the boundaries of the input space.

Taken together, these results validate the use of Gaussian Process Regression for predicting insect syllable rates from morphological and acoustic features. The model's high accuracy, low error margins, and built-in uncertainty estimates make it well-suited for extrapolating to fossil specimens while maintaining biological plausibility.

Results – Syllable rate prediction of fossilised insects

Predicted syllable rates for the nine fossil insect species varied widely across taxa, with corresponding confidence intervals reflecting the degree of uncertainty in each estimate (Extended Data, Fig 8c). Most predictions fell well below their respective biomechanical limits, which were computed as the inverse of syllable duration and served as hard upper bounds on calling rate.

Predicted syllable rates for nine fossil insect species using Gaussian Process Regression. Blue markers indicate the mean predicted syllable rate for each species, with vertical error bars representing standard deviation. Red markers denote the maximum syllable rate for each species.

Archaboilus polyneurus showed a moderate predicted rate ($33.72 \pm 9.64\ s^{-1}$), substantially below its theoretical maximum ($90.91\ s^{-1}$), indicating biologically plausible calling behaviour. In contrast, *Aboilus stratosus* exhibited a lower predicted rate ($8.39 \pm 2.42\ s^{-1}$), yet remained below its biomechanical ceiling ($25.32\ s^{-1}$).

Some taxa exhibited particularly low predicted syllable rates, including *Allaboilus gigantus* ($0.63 \pm 1.08\ s^{-1}$) and *Aboilinae sp. 1* ($3.26 \pm 2.50\ s^{-1}$), likely due to long syllable durations. These predictions, while conservative, were accompanied by relatively small standard deviations, indicating higher model confidence in the low estimates.

Other species such as *Sigmaboilus peregrinus* ($41.38 \pm 32.71\ s^{-1}$) and *Novaboilus ovatus* ($28.78 \pm 13.52\ s^{-1}$) showed wide predictive intervals, suggesting greater extrapolation uncertainty due to feature combinations not well represented in the extant training data. Nevertheless, their mean values remained comfortably below their duration-based limits (133.33 and 62.50 s^{-1}, respectively), upholding biological plausibility.

Across all species, predicted syllable rates remained below their corresponding physical maxima, and the distribution of values reflected both anatomical potential and statistical uncertainty.

Supplementary discussion

Notes on the FEA method

While we lack sufficient data of wing thickness across enough extant taxa to improve the method at this stage, we emphasise that this model was used only to recover the vibrational decay of the wing in response to stimulation in the time domain studies. It also allows for simpler modelling approaches to reduce computation times. The disadvantage is that if some species have unique membrane or vein thicknesses within the vibration region that modify the wing vibration, these are not captured in the model[116].

Relic condition of tegminal stridulation

The relic condition for wing stridulation appears to have started with wings developing sclerotised pegs, randomly distributed on special wing veins, as observed in some cockroaches[117], Mantids[118], in some living Tettigoniidae (e.g. *Pholidoptera* spp.[119]), and in the living Prophalangopsidae, which have traces of vestigial networks of wing veins that exhibit non-functional stridulatory teeth (see Extended Data Fig. 8). Random files struck at the time would produce only broadband calls, as observed in females of many living Tettigoniidae species, which evolved multiple stridulatory veins for duet acoustic response (e.g. many Phaneriopterinae species[5], and in Pseudophyllinae, e.g. *Panoploscelis* spp.[120]). The evolution

of a single stridulatory file, with systematically arranged teeth, is a clear departure or improvement from the relic condition of stridulation.

Wing symmetry and stridulation

Most male Tettigoniidae have asymmetric wings, and have adopted left-over right-wing position, with the left wing bearing the active file, and the right wing the active plectrum. Yet, the earliest Tettigoniidae diverging lineage, the Nedubini, which exhibit symmetric wings, is ambidexterous[121], just like grigs (see below). On the other hand, males of Prophalangopsidae (the grigs, a relic family closely related to modern-day katydids), use symmetric wings and can switch wing overlap during stridulation, just like the earliest diverging Tettigoniidae lineage. Producing pure-tone low frequencies <10 kHz, requires two wings (or two speakers) oscillating in phase to maximise sound output and intensity[122], and this is well studied in field crickets, which use pure tones at ca 5 kHz. The grig *Tarragilus diuturnus* uses similar frequencies, and to the human hear, their calls sound exactly like those of field crickets (Extended Data, Fig. 1). This suggests these species, and their extinct ancestors singing at frequencies below 10 kHz[123], used a wing coupling mechanism (like the field cricket escapement) to synchronise both wings in vibration, and avoid destructive interference to maintain a pure-tone signal[122]. Indication of this symmetrical mechanism could be identified in fossilised wings even if only one wing is found (see Extended Data Figs. 5-6). For example, a single wing with a fully developed file and a fully developed plectrum (designed to scrape a functional file in the opposite wing), suggests that its counterpart likely had the same structures (symmetry) for ambidextrous stridulation.

Bimodal temporal/frequency modulation

A single wing closing phase produces the most basic oscillation of the call, termed here a 'syllable' (Extended Data, Video1). Syllables can be grouped or delivered in sequence to form a repertoire, which includes variations in time and frequency as the main parameters for specific recognition.

In many extant species, a systematic file-tooth distribution can affect the instantaneous frequency and amplitude components of the syllable. This has been described as mechanical frequency modulation (FM) and has been observed in the calls of the leaf mimicking katydids Pterochrozini[124]. This seems to be also a mechanism adopted by some Jurassic Ensifera like *A. polyneurus* (see Extended data, Fig. 7).

Insect ear evolution

Insect ears arise from the specialisation of pre-existing mechanoreceptor organs (precursor organs) that detect substrate vibrations into organs that detect airborne sound[125]. Convincing support for a vibratory origin of the auditory system is given by many interneurons with bimodal vibratory and auditory response properties[126]. Therefore, the ability to detect airborne sounds seems to be an evolutionary addition to the pre-existing adaptation[127]. Importantly, the auditory sensilla precursor to the *cristica acustica* (CA) is found in the complex tibial scolopidial organ of atympanate Ensifera, where the sensilla are specialised in substrate vibration detection. Notable structural changes occurred during the transformation of scolopidial cells specialised in vibrations (low frequencies) to functional sound detectors.

A precursor scolopidium might have started being sensitive to high-amplitude sound before tympana and acoustic trachea appeared. A good example of this condition is preserved in the subgenual organ of some living cockroach species, and the high wing chordotonal organ of locust, both of which have been shown to respond to sound without any tympanal adaptation[128,129]. In this context, sound-capturing structures evolved in successive steps, transforming the cuticle into tympana, and a respiratory trachea into an air space to enhance tympanal vibrations. Subsequently, the tracheal air space backing the tympana was modified into an acoustic trachea or ear canal (Fig. 5a-c), which in modern katydid species focuses on capturing and conducting sounds to the tympanal organ. This gradual transition from vibrational to airborne hearing has been convergently followed in the mammalian transition from jaw bones to the formation of the middle ear ossicles[130]. The timing of this mammalian hearing transition coincides with the specialisation of insectivorous dentition, suggesting that eavesdropping on and predation of singing insects were likely among the driving forces of this transition[131]. The elongation of the cochlear canal to the cochlea of therian mammals was likely constrained and triggered by the coevolution between these small animals and their prey, acoustically active orthopterans[132].

Relic condition of the acoustic trachea

A bifurcated auditory trachea seems to be the plesiomorphic condition of a functional acoustic trachea, as it also occurs in atympanate Ensifera (e.g., Gryllacrididae, see Extended data Fig. 9ab), and is also observed in nymphs during the ontogenetic development of Tettigoniidae species.

Ears have evolved at least 19 times independently in insects. In the period before bats, ears and complex acoustical behaviours appeared independently in at least seven insect orders[126,133,134]. It was recently shown that at least four of the species-rich moth clades (Drepanoidea, Geometroidea, Noctuoidea, and Pyraloidea) evolved ears in the Late Cretaceous (some 103.4 to 67 Ma), several million years before the echolocating bat[135]. The authors suggest that hearing organs in Lepidoptera, and in animals more broadly, evolved to provide auditory surveillance of the environment for broadband sounds produced by potential predators. Behavioural and neural evidence have shown that moths respond to the low-frequency (<20 kHz) walking and wingbeat sounds of predatory birds[136,137]. This suggests that the hearing organs of moths and other insects likely evolved first for auditory surveillance before subsequently being co-opted for bat detection.

References

85. Bolger, A. M., Lohse, M. & Usadel, B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* **30**, 2114–2120 (2014).

86. Luo, R. *et al.* SOAPdenovo2: an empirically improved memory-efficient short-read de novo assembler. *Gigascience* **1**, 2047-217X-1–18 (2012).

87. Petersen, M. *et al.* Orthograph: a versatile tool for mapping coding nucleotide sequences to clusters of orthologous genes. *BMC Bioinformatics* **18**, 111 (2017).

88. Shin, S. *et al.* Orthoptera-specific target enrichment (OR-TE) probes resolve relationships over broad phylogenetic scales. *Sci. Rep.* **14**, 21377 (2024).

89. Katoh, K. & Standley, D. M. MAFFT Multiple Sequence Alignment Software Version 7: Improvements in Performance and Usability. *Mol. Biol. Evol.* **30**, 772–780 (2013).

90. Seabra, S. G. *et al.* Different Genomic Changes Underlie Adaptive Evolution in Populations of Contrasting History. *Mol. Biol. Evol.* **35**, 549–563 (2018).

91. Kozlov, A. M., Darriba, D., Flouri, T., Morel, B. & Stamatakis, A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. *Bioinformatics* **35**, 4453–4455 (2019).

92. Mai, U. & Mirarab, S. TreeShrink: fast and accurate detection of outlier long branches in collections of phylogenetic trees. *BMC Genomics* **19**, 272 (2018).

93. Frandsen, P. B. *et al.* Phylogenomics recovers multiple origins of portable case making in caddisflies (Insecta: Trichoptera), nature's underwater architects. *Proceedings of the Royal Society B: Biological Sciences* **291**, 20240514 (2024).

94. Borowiec, M. L. AMAS: a fast tool for alignment manipulation and computing of summary statistics. *PeerJ* **4**, e1660 (2016).

95. Minh, B. Q. *et al.* IQ-TREE 2: New Models and Efficient Methods for Phylogenetic Inference in the Genomic Era. *Mol. Biol. Evol.* **37**, 1530–1534 (2020).

96. Kalyaanamoorthy, S., Minh, B. Q., Wong, T. K. F., von Haeseler, A. & Jermiin, L. S. ModelFinder: fast model selection for accurate phylogenetic estimates. *Nat. Methods* **14**, 587–589 (2017).

97. Chernomor, O., von Haeseler, A. & Minh, B. Q. Terrace Aware Data Structure for Phylogenomic Inference from Supermatrices. *Syst. Biol.* **65**, 997–1008 (2016).

98. Hoang, D. T., Chernomor, O., von Haeseler, A., Minh, B. Q. & Vinh, L. S. UFBoot2: Improving the Ultrafast Bootstrap Approximation. *Mol. Biol. Evol.* **35**, 518–522 (2018).

99. Hedwig, B. *A Highly Sensitive Opto-Electronic System for the Measurement of Movements*. *Journal of Neuroscience Methods* vol. 100 www.elsevier.com/locate/jneumeth (2000).

100. Montealegre-Z, F. & Mason, A. C. The mechanics of sound production in Panacanthus pallicornis (Orthoptera: Tettigoniidae: Conocephalinae): the stridulatory motor patterns. *Journal of Experimental Biology* **208**, 1219–1237 (2005).

101. Celiker, E. *et al.* Mechanical network equivalence between the katydid and mammalian inner ears. *PLoS Comput. Biol.* **20**, e1012641 (2024).

102. Rasmussen, C. E. & Williams, C. K. I. *Gaussian Processes for Machine Learning*. (MIT Press, Cambridge, MA, 2006).

103. Whitesell, J. J. & Walker, T. J. Photoperiodically determined dimorphic calling songs in a katydid. *Nature* **274**, 887–888 (1978).

104. Walker, T. J., Whitesell, J. J. & Alexander, R. D. The robust conehead: two widespread sibling species (Orthoptera: Tettigoniidae: Neoconocephalus 'robustus'). *Ohio Journal of Science* **73**, 321–330 (1973).

105. Walker, T. J., Forrest, T. G. & Spooner, J. D. The rotundifolia complex of the genus Amblycorypha (Orthoptera: Tettigoniidae): songs reveal new species. *Ann. Entomol. Soc. Am.* **96**, 433–447 (2003).

106. Walker, T. J. The uhleri group of the genus Amblycorypha (Orthoptera: Tettigoniidae): extraordinarily complex songs and new species. *J. Orthoptera Res.* **13**, 169–183 (2004).

107. Walker, T. J. Systematics and acoustic behavior of United States crickets of the genus Cyrtoxipha (Orthoptera: Gryllidae). *Ann. Entomol. Soc. Am.* **62**, 945–952 (1969).

108. Walker, T. J. Systematics and acoustic behavior of United States crickets of the genus Orocharis (Orthoptera: Gryllidae). *Ann. Entomol. Soc. Am.* **62**, 752–762 (1969).

109. Walker, T. J. The taxonomy and calling songs of United States tree crickets (Orthoptera: Gryllidae: Oecanthinae). *Ann. Entomol. Soc. Am.* **55**, 303–322 (1962).

110. Prestwich, K. N. & Walker, T. J. Energetics of singing in crickets: effect of temperature in three trilling species (Orthoptera: Gryllidae). *Journal of Comparative Physiology B* **143**, 199–212 (1981).

111. Morris, G. K. & Gwynne, D. T. Geographical distribution and biological observations of Cyphoderris (Orthoptera: Haglidae) with a description of a new species. *Psyche (Stuttg).* **85**, 147–167 (1978).

112. Scotese, C. R. An atlas of Phanerozoic paleogeographic maps: the seas come in and the seas go out. *Annu. Rev. Earth Planet. Sci.* **49**, 669–718 (2021).

113. Jenkyns, H. C., Schouten-Huibers, L., Schouten, S. & Sinninghe Damsté, J. S. Warm Middle Jurassic-Early Cretaceous high-latitude sea-surface temperatures from the Southern Ocean. *Climate of the Past* **8**, 215–225 (2012).

114. Chen, L. *et al.* Reconstructing fossil insect communities and palaeoclimates for the Middle Jurassic Yanliao Biota in the Daohugou area of China. *Sci. Bull. (Beijing).* https://doi.org/10.1016/j.scib.2024.11.010 (2024) doi:10.1016/j.scib.2024.11.010.

115. Akiba, T., Sano, S., Yanase, T., Ohta, T. & Koyama, M. Optuna: A Next-generation Hyperparameter Optimization Framework. *Proceedings of the ACM SIGKDD International Conference on Knowledge Discovery and Data Mining* 2623–2631 (2019) doi:10.1145/3292500.3330701.

116. Gaiddon, T. *et al.* The harmonic-dominant signals in animal communication involve the use of new resonant frequencies. *Heliyon* **12**, e44424 (2026).

117. Roth, L. M. & Hartman, H. B. Sound Production and Its Evolutionary Significance in the Blattaria. *Ann. Entomol. Soc. Am.* **60**, 740–752 (1967).

118. Hill, S. A. Sound generation in Mantis religiosa (Mantodea: Mantidae): stridulatory structures and acoustic signal. *J. Orthoptera Res.* **16**, 35–49 (2007).

119. Heller, K.-G. *Bioakustik Der Europäischen Laubheuschrecken*. (Verlag Josef Margraf, 1988).

120. Hugel, S. Panoploscelis scudderi Beier, 1950 and Gnathoclita vorax (Stoll, 1813): two katydids with unusual acoustic, reproductive and defense behaviors (Orthoptera, Pseudophyllinae). *Zoosystema* **40**, 327–340 (2019).

121. Cole, J. A. *et al.* A revision of the shield-back katydid genus Neduba (Orthoptera: Tettigoniidae: Tettigoniinae: Nedubini). *Zootaxa* **4910**, 1–92 (2021).

122. Jonsson, T., Montealegre-Z, F., Soulsbury, C. D. & Robert, D. Tenors Not Sopranos: Bio-Mechanical Constraints on Calling Song Frequencies in the Mediterranean Field-Cricket. *Front. Ecol. Evol.* **9**, 647786 (2021).

123. Woodrow, C., Baker, E., Jonsson, T. & Montealegre-Z, F. Reviving the sound of a 150-year-old insect: The bioacoustics of Prophalangopsis obscura (Ensifera: Hagloidea). *PLoS One* **17**, e0270498 (2022).

124. Montealegre-Z, F. Biomechanics of musical stridulation in katydids (Orthoptera: Ensifera: Tettigoniidae): an evolutionary approach. (2005).

125. Yager, D. D. Structure, development, and evolution of insect auditory systems. *Microsc. Res. Tech.* **47**, 380–400 (1999).

126. Kalmring, K., Sickmann, T., Jatho, M., Zhantiev, R. & Grossbach, M. The auditory-vibratory sensory system of Polysarcus denticauda (Phaneropterinae, Tettigoniidae) 3. Physiology of the ventral cord neurons ascending to the head ganglia. *Journal of Experimental Zoology* **279**, 9–28 (1997).

127. Strauß, J. & Lakes-Harlan, R. Evolutionary and Developmental Adaptations of Tympanal Receptor Organs in Insects. in *Insect Hearing and Acoustic Communication* (ed. Hedwig, B.) 5–26 (Springer Berlin Heidelberg, 2014). doi:10.1007/978-3-642-40462-7_2.

128. Shaw, S. R. Detection of airborne sound by a cockroach 'vibration detector': a possible missing link in insect auditory evolution. *Journal of Experimental Biology* **193**, 13–47 (1994).

129. Pearson, K. G., Hedwig, B. & Wolf, H. Are the Hindwing Chordotonal Organs Elements of the Locust Flight Pattern Generator. *Journal of Experimental Biology* **144**, 235–255 (1989).

130. Mao, F. *et al.* Fossils document evolutionary changes of jaw joint to mammalian middle ear. *Nature* **628**, 576–581 (2024).

131. Mao, F. *et al.* Jurassic shuotheriids show earliest dental diversification of mammaliaforms. *Nature* **628**, 569–575 (2024).

132. Rodrigues, P. G., Ruf, I. & Schultz, C. L. Digital Reconstruction of the Otic Region and Inner Ear of the Non-Mammalian Cynodont Brasilitherium riograndensis (Late Triassic, Brazil) and Its Relevance to the Evolution of the Mammalian Ear. *J. Mamm. Evol.* **20**, 291–307 (2013).

133. Hoy, R. R. The Evolution of Hearing in Insects as an Adaptation to Predation from Bats. in *The Evolutionary Biology of Hearing* (eds. Webster, D. B., Fay, R. R. & Popper, A. N.) 115–129 (Springer-Verlag, 1992). doi:10.1007/978-1-4612-2784-7_5.

134. Yack, J. E. The structure and function of auditory chordotonal organs in insects. *Microsc. Res. Tech.* **63**, 315–337 (2004).

135. Kawahara, A. Y. *et al.* Phylogenomics reveals the evolutionary timing and pattern of butterflies and moths. *Proceedings of the National Academy of Sciences* **116**, 22657–22663 (2019).

136. Jacobs, D. S., Ratcliffe, J. M. & Fullard, J. H. Beware of bats, beware of birds: the auditory responses of eared moths to bat and bird predation. *Behavioral Ecology* **19**, 1333–1342 (2008).

137. Fournier, J. P., Dawson, J. W., Mikhail, A. & Yack, J. E. If a bird flies in the forest, does an insect hear it? *Biol. Lett.* **9**, 20130319 (2013).

138. Vincent, J. F. V. & Wegst, U. G. K. Design and mechanical properties of insect cuticle. Arthropod Struct. Dev. 33, 187-199 (2004).

139. Jafarpour, M., Eshghi, S., Darvizeh, A., Gorb, S. & Rajabi, H. Functional significance of graded properties of insect cuticle supported by an evolutionary analysis. J. Royal Soc. Interface 17, 20200378 (2020).

Supplementary notes

Funding

This research was founded by: the National Key R&D Program of China, grant no. 2025YFF0811700 to DoR; the National Natural Science Foundation of China, grant no. 42372013 to JJG; the Leverhulme Trust, UK, grant no. RPG-2014-284 to FM-Z and DaR; grant RPG-2023–204 to FM-Z. European Research Council (ERC) grant Grant ERCCoG-2017–773067 to FM-Z; FMZ & HS were also supported by a U.S.A. & UK collaborative grant National Science Foundation and Natural Environment Research Council [NERC] (grant DEB-1937815).

Acknowledgement

We thank Prof. Greg Sutton, and Shannon Harrison, University of Lincoln for allowing us to use their High-Speed video camera to record some of the singing insects studied here.

Author contributions

Conceptualization: F. M-Z., J-J. G., J. T., F. S., D. R., & D. Ren;
Methodology: F. M-Z., J. T., C. W., E. C., M. N., J. B. L., H. S., D. R. & J-J. G.;
Software: F. M-Z., J. T., C. W., E. C., & M. N.
Validation: J-J. G., F. M-Z., J. T., C. W., E. C., M. N., J. B. L., F. S-S., F. S., H. S., D. R., & D. Ren;
Formal analysis: F. M-Z., J. T., J-J. G., C. W., E. C., M. N., J. B. L., F. S-S., H. S.;
Investigation: F. M-Z., J. T., J-J. G., C. W., E. C., M. N., J. B. L., F. S-S. & D.R.;
Resources: F. M-Z., H. S., D. R., J-J. G., & D. Ren;
Data curation: J-J. G., F. M-Z., J. T., C. W., E. C., M. N., J. B. L., & H. S.;
Writing - Original Draft: F. M-Z., J. T., J-J. G., C. W., E. C., & D. R.;
Writing - review & editing: F. M-Z., J. T., C. W., E. C., J-J. G., M. N., J. B. L., F. S-S., H. S., D. R., & D. Ren;
Visualisation: J-J. G., F. M-Z., J. T., C. W., E. C., M. N., & J. B. L.;
Supervision: F. M-Z., H. S., D. R., & D. Ren;
Administration: F. M-Z., J-J. G., F. S., D. R., & D. Ren;
Funding acquisition: J-J. G., F. M-Z., H. S., D. R., & D. Ren.

Competing Interests declaration

The authors declare not competing interest.

542

```
MATLAB CODE FOR CALL RECONSTRUCTION BASED ON EXCITATION OF A FOSSILISED
WING RECOVERED IN 2D

%%%%%%%%%%%%%%%%%% Syllable Generation
%%%%%%%%%%%%%%%%%%%%%%%%%%

wav = readmatrix('COMSOL_vibration file.txt');

wavdt = detrend(wav(:,2));


%[b,a] = butter(9,3000/100000,'high');

%wavbut = filter(b,a,wav(:,2))


dt = readmatrix('DeltaT_vector.txt');

x = zeros(length(wav(:,2))+length(dt)*ceil(mean(dt*2e5)),1);

deltat = 0;

lastdt = 0;

x(1:length(wav(:,2))) = x(1:length(wav(:,2)));


for i = 1:length(dt)

lastdt = deltat;

deltat = deltat + round(dt(i)*1e5);

x(1+deltat:deltat+length(wav(:,2))) = x(1+deltat:deltat+length(wav(:,2))) + wavdt;

end
```

```
565 figure
566 plot(x)
567
568 %%%%%%%%%%%%%%%%%%%%%%%% FFT
569 %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
570
571 p=nextpow2(length(x));  %%%
572 Ln=2^(p-1);
573 fs=1/wav(2,1);
574 dt = 1/fs;
575 t = 0:dt:(Ln*dt)-dt;
576
577 f = x;
578
579 frq = (fs/Ln)*(0:Ln/2);
580 f = detrend(f);
581 fft_dat=fft(f(1:Ln));  %%%
582
583 fft_dat2=fft_dat;
584 threshold = max(abs(fft_dat)/Ln)/10000;
585 fft_dat2(abs(fft_dat)/Ln<threshold) = 0;
586
```

```
587     mag_dat = 2*abs(fft_dat(1:Ln/2+1)/Ln);
588     phase_dat = unwrap(angle(fft_dat2(1:Ln/2+1)/Ln));
589
590     figure
591     %subplot(2,1,1)
592     plot(frq(50:4096)/1000,mag_dat(50:4096),'r','LineWidth',1.5)
593     xlabel('Frequency (kHz)')
594     ylabel('Magnitude (Pa)')
595     title('Frequency Decomposition')
596     %axis([0 64 0 110])
597     grid on
598
599
600
```