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Phylogenomics resolves the century-old 'Zoraptera problem': Zoraptera as the earliest diverging lineage of Polyneoptera

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Wing evolution enabled insects to pioneer aerial niches, a key innovation driving their remarkable radiation. However, interordinal relationships among early-diverging winged insects remain contentious, largely owing to the enigmatic phylogenetic position of Zoraptera, a species-poor and morphologically cryptic order. Since its discovery over a century ago, the 'Zoraptera problem' has persisted as a crucial issue in insect evolution, as alternative placements imply fundamentally different scenarios for polyneopteran diversification and trait evolution. Here, we sequenced and assembled two zorapteran genomes, integrating them with public data to compile a dataset of 89 polyneopteran species and 23 outgroups. Using 1367 universal single-copy orthologous genes, we evaluated phylogenetic signal under different evolutionary models and data treatments. Model testing revealed that the prevailing Haplocercata-first hypothesis (Zoraptera + Dermaptera as the earliest polyneopteran branch) collapses when among-site compositional heterogeneity is modelled to reduce systematic errors. Critically, under the optimal site-heterogeneous model, Zoraptera emerges as the earliest-diverging polyneopteran lineage, with Dermaptera + Plecoptera forming the sister clade Dermoplectopterida to all other Polyneoptera, a topology corroborated by multispecies coalescent and gene jackknifing analyses. Our findings highlight the necessity of multi-model approaches in insect phylogenomics, providing a robust framework for early winged insect diversification.

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1. Introduction

The ability to fly paved the way for the winged insects (Pterygota) to become the most diverse and abundant animal group, and today pterygote insects account for over 98% of hexapod species richness [1–3]. However, the interordinal relationships within Polyneoptera, an ancient and diverse clade of winged insects with a fossil record extending to the Carboniferous, remain unclear [4–10]. Polyneoptera includes the first great diversification of neopteran insects, which are winged insects with the novel ability to fold their wings over the body at rest. This ability protects their wings when not in use and also allows them to take on many novel secondary specializations [1,2]. Fossil evidence indicates that Polyneoptera constitutes the first major proliferation of insect lineages [1,11,12], with many groups remaining significant components of the insect fauna to this day. The evolutionary roots of the eleven extant polyneopteran orders (earwigs, grasshoppers, mantises, roaches, stick insects and allies) have proven difficult to resolve [5,13–15]. Uncertainties in polyneopteran relationships have implications for our understanding of the early diversification of neopteran winged insects since reconstructing the morphology and biology of the ancestral polyneopteran is contingent on resolving the early divergences in the clade.

Crucial to disentangling the early evolution of Polyneoptera is the phylogenetic position of the enigmatic order Zoraptera, sometimes referred to as 'groundlice' or 'angel insects'. Zorapterans are typically diminutive insects that exhibit a superficial resemblance to termites or barklice and are characterized by their gregarious behaviour (figure 1). With less than 50 described extant species across 10 established genera, zorapterans are the third smallest and arguably the least understood insect order [16]. Since their discovery in 1913 [17], the phylogenetic position of zorapterans has represented one of the most persistent problems in reconstructing the insect Tree of Life. They have been placed in a bewildering number of positions, including as sister group to all Holometabola (insects that undergo full metamorphosis), Acercaria (Hemiptera and allies), Dictyoptera (Mantodea+Blattodea), Eukinolabia (Phasmatodea+Embiodea), webspinners (Embiodea), stoneflies (Plecoptera) and earwigs (Dermaptera) [18–27]. Over the past decade, phylogenetic analyses of morphological and molecular data have unequivocally supported the placement of zorapterans within Polyneoptera, but systematic affinities with almost all polyneopteran clades have been suggested (e.g. [5,6,9,16,23,28–30]), leading researchers [25] to dub this the 'Hundred-Year Conundrum', reflecting its resistance to resolution. The Zoraptera problem results, at least in part, from the insects' unusual biology and associated morphological specializations. The minute adults occur as either fully sighted (i.e. with well-developed compound eyes) alate individuals or blind and wingless morphs that live in colonies inside decaying logs or under stones [31] (figure 1). Aside from their unusual morphology and high rates of molecular evolution [25], incongruences in resolving the systematic placement of zorapterans are exacerbated by their scant fossil record, which discloses little about their affinities [32].

To resolve the phylogenetic position of Zoraptera, we sequenced the genomes of two zorapteran genera and integrated public data to achieve comprehensive and balanced taxon sampling across all major polyneopteran lineages. This yielded the most species-rich phylogenomic dataset to date, overcoming previous limitations in zorapteran representation. Through extensive testing of evolutionary models, comparison of phylogenies using different procedures and statistical assessments to mitigate systematic biases, we identified key factors influencing the placement of Zoraptera and recovered a robust polyneopteran phylogeny.

2. Material and methods

To resolve the long-standing uncertainty surrounding the phylogenetic placement of Zoraptera within Polyneoptera (figure 2B), we generated novel genome assemblies for *Latinozoros* sp. and *Brazilozoros* sp. (figures 1B,C and 2C). These were integrated with three previously published zorapteran transcriptomes, collectively providing representative coverage across both recognized families and three of the four extant subfamilies of Zoraptera (figure 2A; electronic supplementary material, table S1). In addition, we assembled publicly available genomic and transcriptomic data from 84 additional polyneopteran species, encompassing all 10 extant orders, as well as 23 outgroup taxa representing Acercaria, Holometabola, Palaeoptera, Zygentoma, and Archaeognatha (figure 2A; electronic supplementary material, table S1). Universal single-copy orthologues were identified, aligned, trimmed and concatenated into six amino-acid supermatrices differing in taxon occupancy and evolutionary-rate filters (electronic supplementary material, figure S1; table S1). Phylogenetic relationships were inferred using both multispecies coalescent (MSC) and concatenation-based frameworks under a comprehensive suite of substitution models, encompassing partitioned homogeneous models, finite mixture models (LG4X+R, LG+C20+F+G, LG+C60+F+G), the infinite mixture CAT-GTR model and posterior mean site-frequency approximations (C60-PMSF and CAT-PMSF) (figure 3 and electronic supplementary material, figure S2). Model performance and systematic biases were rigorously evaluated through cross-validation, posterior predictive checks, topology tests and jackknife resampling (figures 4 and 5; electronic supplementary material, figure S2; tables S2 and S3). Targeted subsampling of slowly evolving loci further demonstrated that gene evolutionary rate critically influences topological congruence (electronic supplementary material, figure S3). This integrated, multi-model approach provides a robust assessment of both data quality and model adequacy, enabling confident resolution of deep-branching relationships within early-diverging Polyneoptera (electronic supplementary material, figure S4). For detailed information regarding the specific analytical procedures, software and associated parameters, refer to the online electronic supplementary material.

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Figure 1. Zorapteran species and their habitats. (A) *Centrozoros gurneyi* (apterous female), Laguna de Volcán, Volcán, Chiriquí Province, Panama. (B) *Latinozoros cacaoensis* (apterous male), Sentier du Bagne des Annamites track, Montsinery, French Guiana. (C) *Brazilozoros* cf. *huxleyi* (alate and dealate females), Montagne des Singes, Kourou, French Guiana. (D) Habitat of an unidentified *Centrozoros* sp. in Mount Totumas Cloud Forest, Los Pozos, Chiriquí Province, Panama. (E) Habitat of *Latinozoros cacaoensis* and *Brazilozors* sp. at the Sentier du Bagne des Annamites track, Montsinery, French Guiana.

3. Results

(a) The placement of Zoraptera is model dependent

We constructed two focal matrices based on taxon occupancy: matrix 1 comprises 278 360 amino acid (AA) sites across 1049 loci, while matrix 2 contains 131 898 AA sites spanning 527 loci. All analyses based on the primary matrices strongly support the monophyly of Polyneoptera (SH-like approximate likelihood ratio test (SH-aLRT) = 100, ultrafast bootstrap approximation (UFBoot) = 100, Bayesian posterior probability (BPP) = 1; electronic supplementary material, figures S2 and S3). However, the phylogenetic relationships among the early-diverging lineages, Zoraptera, Dermaptera and Plecoptera, vary depending on the evolutionary models employed (figure 3; electronic supplementary material, table S2). Under the homogeneous partitioned model, Zoraptera and Dermaptera form a well-supported sister clade (SH-aLRT = 100, UFBoot = 100), with Haplocercata representing the sister group to the remaining Polyneoptera (figure 3, electronic supplementary material, figures S2A,B). Intriguingly, when examining the results under the mixture models, finite mixture models (LG4X+R, LG+C20+F+G and LG+C60+F+G) also recover the Haplocercata clade, but with progressively declining support (SH-aLRT = 89.9–100.0 $_{[matrix1]}$ and 41.1–100.0 $_{[matrix2]}$, UFBoot = 80–100 $_{[matrix1]}$ and 61–100 $_{[matrix2]}$) as model complexity (i.e. number of categories) increases (figure 4A and electronic supplementary material, figures S2C–K).

By contrast, analyses employing infinite mixture models (CAT-PMSF and CAT-GTR) strongly favour an alternative topology, placing Zoraptera as the sister lineage to all other polyneopterans (SH-aLRT = 100, UFBoot = 100, BPP = 1; figure 3 and electronic supplementary material, figures S2L–P), while Dermaptera and Plecoptera form a highly supported clade (SH-aLRT = 100, UFBoot = 100, BPP = 1; figure 3 and electronic supplementary material, figures S2L–P). This arrangement is further corroborated by our coalescent analyses under the MSC model (figure 5B, electronic supplementary material, figures S2R,S). In analyses under the C60-PMSF model, using the two competing topological hypotheses as guide trees, both competing hypotheses were supported, albeit with weaker nodal support (SH-aLRT = 89.9 $_{[matrix1]}$ and 81.8$_{[matrix2]}$, UFBoot = 80 $_{[matrix1]}$ and 83$_{[matrix2]}$) for the Haplocercata-first hypothesis (figures 3 and 4A, and electronic supplementary material, figures S2G–J). These results demonstrate that PMSF-based inferences are sensitive to guide tree selection.

The relationships among the remaining polyneopteran orders are stable over all models: Orthoptera are sister group to the remaining Polyneoptera (excluding Zoraptera, Dermaptera and Plecoptera), Mantophasmatodea+Grylloblattodea (Notoptera) form a sister clade to Phasmatodea+Embiodea (Eukinolabia), collectively termed Mecynoptera, and Mantodea are sister group to Blattodea (Dictyoptera) (figure 3 and electronic supplementary material, figure S2).

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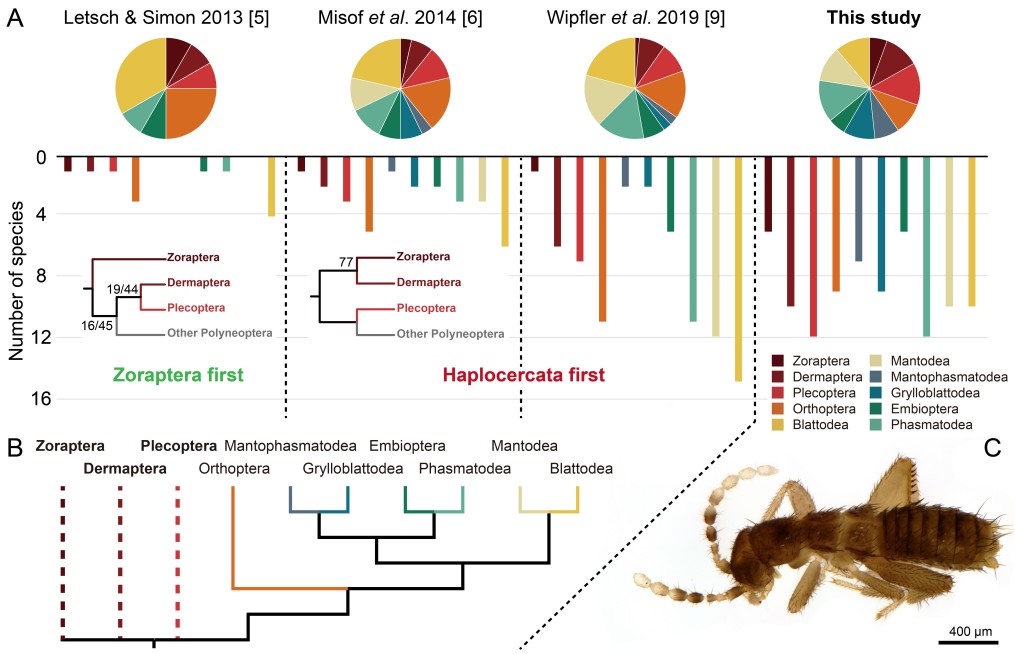


Figure 2. Current status of phylogenomic studies on Polyneoptera. (A) Taxon sampling and topological hypotheses of basal polyneopterans. Pie charts illustrate the proportional representation of each polyneopteran order in the sampled datasets, while bar graphs display the absolute number of sampled species per order. The basal relationships of Polyneoptera presented here are derived from the primary results of their respective original study [5,6,9]; nodes with unresolved or weakly supported relationships are annotated with their respective support values. (B) Polyneopteran phylogeny based on current knowledge. (C) *Latinozoros* sp. (Zoraptera), representing a sequenced genus in this study.

(b) Model comparison and topology test

Tree reconstruction artefacts commonly emerge when evolutionary models rely on incorrect assumptions about the data [33,34]. In our analyses, the Haplocercata-first and Zoraptera-first hypotheses were preferentially supported under homogeneous partition models and heterogeneous CAT-based models, respectively (figure 3B). Notably, support for the Haplocercata-first hypothesis diminished as the complexity of the heterogeneity model increased, highlighting the sensitivity of phylogenetic inference to model selection (figure 4A). This observation prompted a formal model comparison to assess predictive performance and goodness-of-fit, thereby guiding the judgement of the most reliable phylogenetic hypothesis.

The CAT-GTR model outperformed both the LG+C60+G and LG+C20+G models, as demonstrated by LOO-CV and widely applicable information criterion (wAIC). Specifically, the CAT-GTR model showed improved scores (ΔCV = $-47.5703_{[\text{CAT-GTR}]} + 48.8538_{[\text{LG+C60+G}]} = 1.2835$ and $-47.5703_{[\text{CAT-GTR}]} + 49.1333_{[\text{LG+C20+G}]} = 1.563$; ΔwAIC = $-47.5399_{[\text{CAT-GTR}]} + 48.8538_{[\text{LG+C60+G}]} = 1.3139$ and $-47.5399_{[\text{CAT-GTR}]} + 49.1332_{[\text{LG+C20+G}]} = 1.5933$), indicating superior predictive accuracy (figure 4B; electronic supplementary material, table S3). Posterior predictive checking assessed model adequacy, with *p*-values of 0 (indicating complete misfit under finite Markov chain Monte Carlo simulations) for all models, suggesting systematic model misspecification. However, the severity of misfit varied substantially, as quantified by *Z*-scores measuring deviations between observed and predicted site patterns (figure 4B). The LG+C60+G and LG+C20+G models showed extreme discrepancies (Z = 66.76 and 99.32, respectively), reflecting severe failure to capture empirical data structure (figure 4B; electronic supplementary material, table S3). By contrast, the CAT-GTR model exhibited significantly lower (though still substantial) deviation (Z = 23.31), indicating its comparatively better, albeit imperfect, fit to the data (figure 4B; electronic supplementary material, table S3).

Furthermore, the evaluation of model performance under maximum likelihood (ML) framework revealed consistent superiority of site-heterogeneous models (figure 4A; electronic supplementary material, table S3). Analysis of AIC, BIC and log-likelihood values demonstrated that the partitioned model exhibited inferior fit compared with both LG+C20+F+G and LG+C60+F+G models across all matrices examined (figure 4A; electronic supplementary material, table S3). Based on these findings, we treated the topology reconstructed under the CAT-based models as the most reliable phylogenetic framework.

To enhance the robustness of alternative hypothesis evaluation, we further conducted topology tests under a ML framework using two complex site-heterogeneous models, LG+C60+F+G and CAT-PMSF, in IQ-TREE to examine competing topologies. While the LG+C60+F+G model failed to reject either hypothesis statistically , the CAT-PMSF analysis provided decisive discriminatory power (table 1). The CAT-PMSF results robustly rejected the Haplocercata-first hypothesis favoured by analyses under site-homogeneous or suboptimal site-heterogeneous models, conversely providing maximal support for the Zoraptera-first topology recovered in CAT-based and coalescent analyses (table 1).

(c) Jackknife analyses

Phylogenetic analyses using the best-fit CAT-GTR model for large datasets (>100 taxa, >50 000 aligned positions) proved computationally expensive and struggled to achieve statistical convergence criteria. We therefore implemented jackknife

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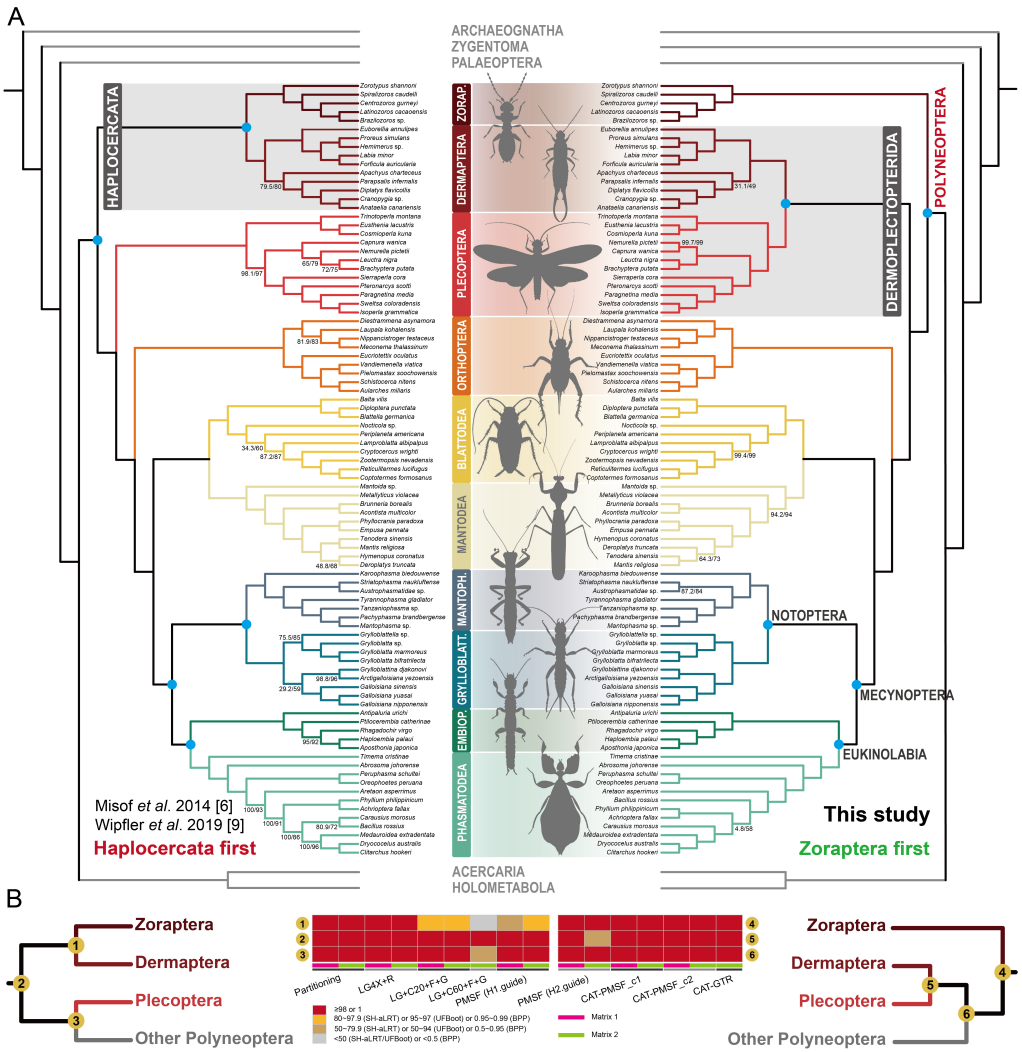


Figure 3. Impact of compositional heterogeneity across sites on polyneopteran phylogeny. (A) Competing topological hypotheses: the Haplocercata-first topology (supported by Misof *et al.* [6] and Wipfler *et al.* [9]) was derived from the analysis with the site-homogeneous partitioned model using matrix 2, whereas the Zoraptera-first topology was obtained from the analysis of matrix 2 under the site-heterogeneous CAT-PMSF model. All nodes except those marked with black numerals (SH-aLRT/UFBoot) received maximum nodal support. Silhouettes: *Deinacrida rugosa* (Orthoptera) by Melissa Broussard (PhyloPic; CC BY 3.0); Zoraptera, Dermaptera, Mantophasmatodea, Embioptera and Phasmatodea under CC0 1.0 Universal; others generated in this study. (B) Summary of phylogenetic analyses under multiple models, with support values for focal nodes indicated by coloured squares (for maximum likelihood (ML) analyses, square colour is determined by the lower support value between SH-aLRT and UFBoot). Abbreviations: Embiop., Embioptera; Grylloblatt., Grylloblattodea; Mantoph., Mantophasmatodea; Zorap., Zoraptera; BPP, Bayesian posterior probability; SH-aLRT and UFBoot, node supports from SH-like approximate likelihood ratio tests and ultrafast bootstrap approximation.

resampling procedures for the largest matrix 1 analysis. The results showed maximal support (jackknife proportions [JPs] = 100) for Zoraptera as the earliest-diverging lineage within Polyneoptera, while the sister-group relationship between Dermaptera and Plecoptera received strong support (JPs = 97), with only a single subset analysis recovering these orders as the second and third diverging branches, respectively (figure 5A and electronic supplementary material, figure S2Q). Collectively, the jackknife analyses provided compelling support for the Zoraptera-first hypothesis.

(d) Impact of data filtering on the recovered topologies

To assess the impact of gene evolutionary rates on phylogenetic reconstruction, we created four additional matrices by applying increasingly stringent average pairwise identity (API) thresholds (>0.5, 0.6, 0.7 and 0.8). These datasets comprise: (i) matrix 3 with 368 533 AA sites from 1268 loci, (ii) matrix 4 with 275 833 AA sites from 936 loci, (iii) matrix 5 with 1 25 265 AA sites from 469 loci and (iv) matrix 6 with 41 584 AA sites from 181 loci.

We performed ML analyses using the LG4X+R model and coalescent analyses under the MSC model across all matrices (figure 5B and electronic supplementary material, figures S3A–F,I,J; table S2). All coalescent analyses consistently recovered the Zoraptera-first topology, concordant with results from primary matrices. By contrast, LG4X+R analyses produced conflicting results: while matrices 3 and 4 supported the Haplocercata-first hypothesis (consistent with primary matrix LG4X+R results but rejected by prior topology tests), analyses of matrices 5 and 6 favoured the Zoraptera-first hypothesis (figure 5B). Follow-up examinations of matrices 5 and 6 using both site-homogeneous partitioned models and the site-heterogeneous LG+C20+F+G model unanimously supported the Zoraptera-first hypothesis (figure 5B and electronic supplementary material,

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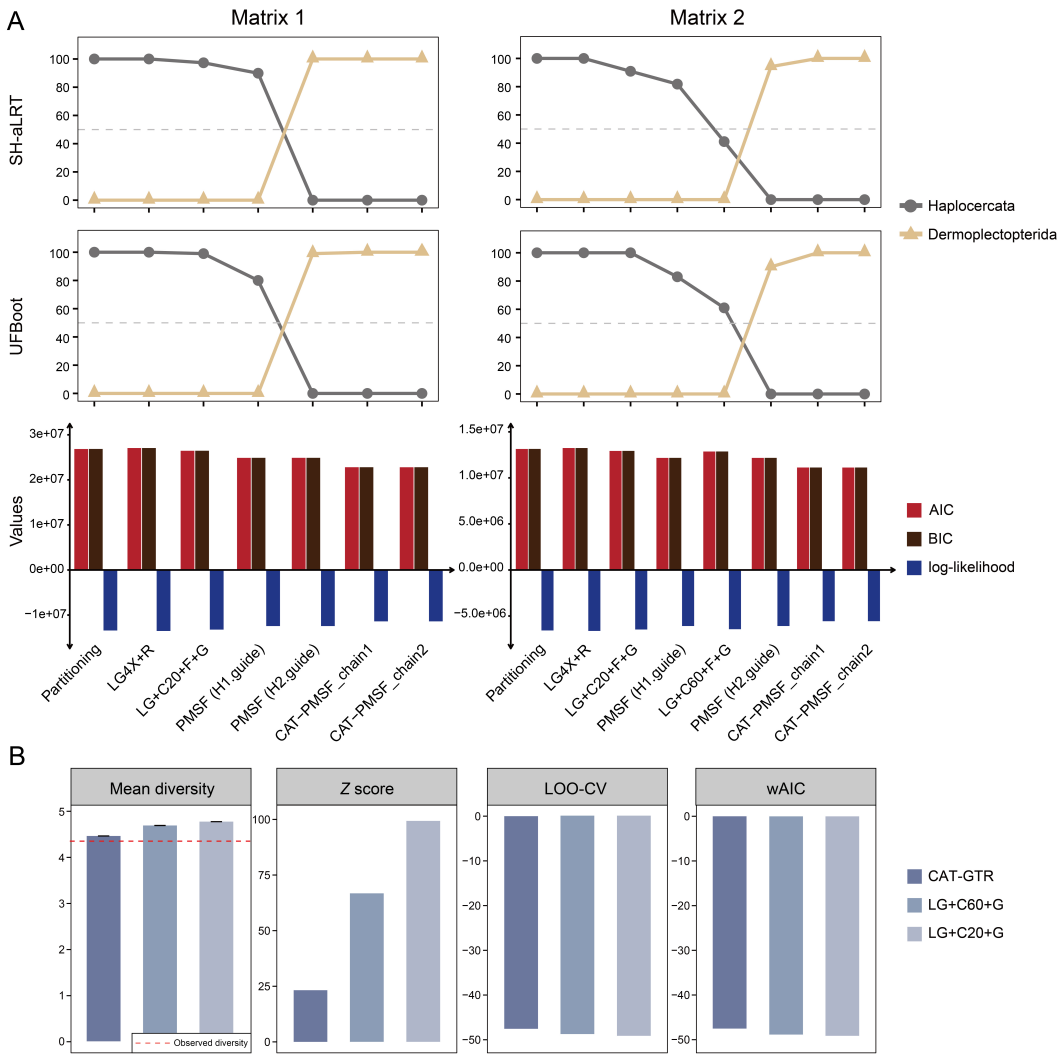


Figure 4. Changes in support for alternative clades and comparisons of model fit. (A) Variation in support values for Haplocercata and Dermoplectopterida under increasing model complexity, based on analyses of matrices 1 and 2 within the ML framework. Also shown are model performance metrics, including AIC, BIC and log-likelihood. (B) Model fit assessments under the Bayesian framework using PhyloBayes. Abbreviations: Mean diversity refers to the average predicted number of distinct amino acid residues per site; LOO-CV, leave-one-out cross-validation score; wAIC, widely applicable information criterion from Phylobayes analysis; AIC, Akaike information criterion scores from the IQ-TREE analysis; BIC, Bayesian information criterion scores from IQ-TREE analysis; SH-aLRT and UFBoot represent node support values from SH-like approximate likelihood ratio tests and ultrafast bootstrap approximation, respectively.

figures S3G,H,K,L). These results establish gene evolutionary rate as a critical factor influencing Polyneoptera phylogenetic reconstruction. Datasets filtered using API thresholds of 0.6 and 0.8 demonstrated superior compatibility with current evolutionary models, yielding congruent results across site-homogeneous, site-heterogeneous and MSC frameworks (figure 5B).

Collectively, our findings demonstrate that the phylogenetic placement of Zoraptera is fundamentally contingent upon two critical determinants: (i) implementation of better-fitting substitution models and (ii) selection of genes with slower evolutionary rates.

4. Discussion

(a) The phylogeny of Polyneoptera

The phylogenetic relationships among Polyneoptera have long been enigmatic. Early phylogenetic analyses based on morphological data and Sanger sequences yielded diverse and often conflicting hypotheses [25]. The advent of phylogenomic approaches has shed new light on polyneopteran phylogeny. In particular, the relationships among the polyneopteran orders, excluding the early-diverging lineages Zoraptera, Dermaptera and Plecoptera, have been consistently recovered in previous transcriptome-based studies [5,6,9,35] (figure 2B). This topology among the 'core Polyneoptera' was again confirmed in our study, demonstrating a robust evolutionary signal in shallower divergences. However, the relative positions of Zoraptera, Dermaptera and Plecoptera remain unstable across different datasets and analytical approaches [5,6,9,35] (figure 2A,B). Zoraptera represents a typical fast-evolving lineage, exhibiting a conspicuously long branch subtending the rest of the taxa in prior analyses [36–38], which suggests that its phylogenetic placement is particularly susceptible to reconstruction artefacts. Notably, Zoraptera is recovered as sister group to Dermaptera (Haplocercata-first) only under suboptimal models, including

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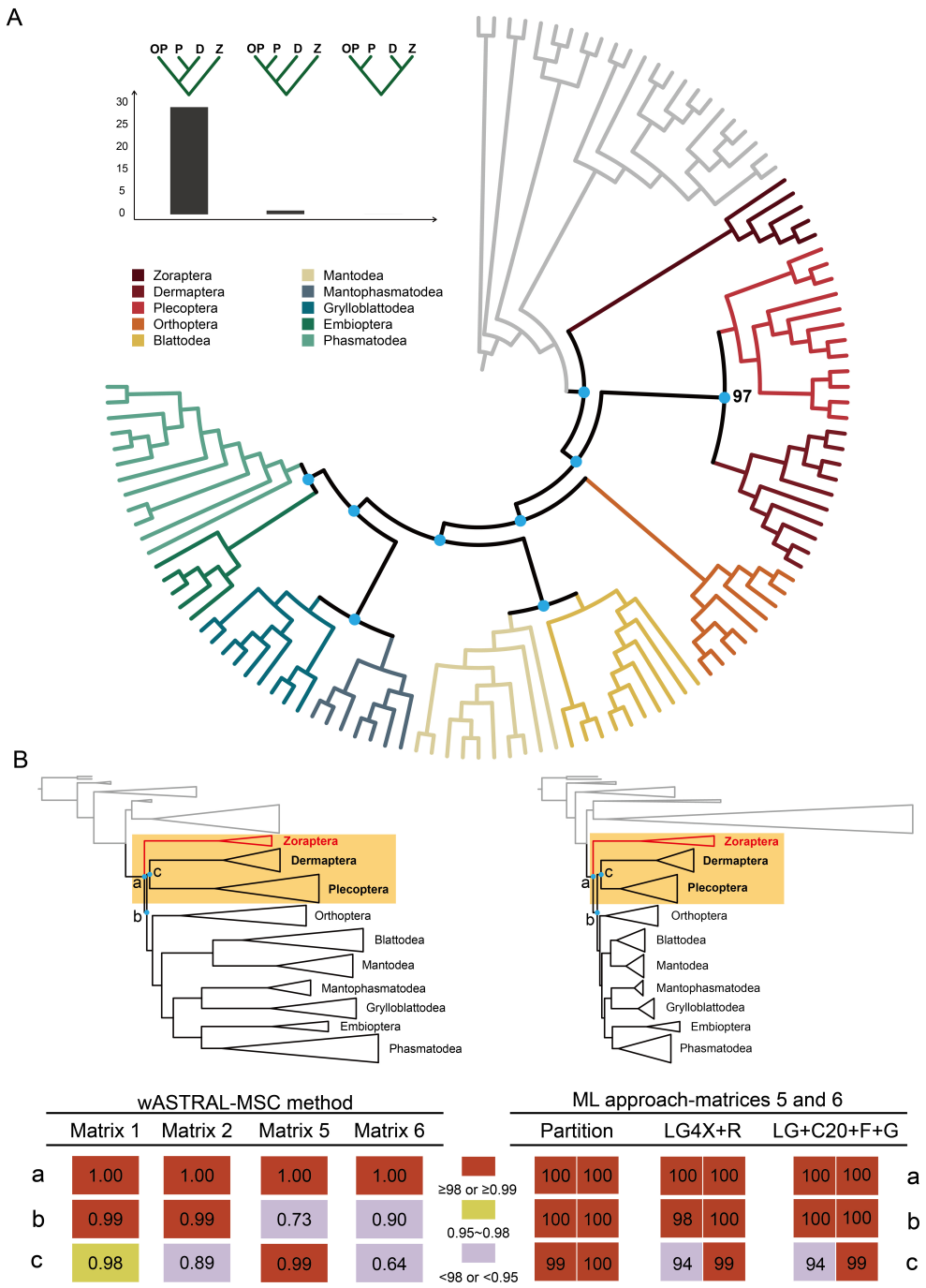


Figure 5. Additional lines of evidence supporting the Zoraptera-first hypothesis. (A) Polyneopteran phylogeny inferred from matrix 1 using a jackknife resampling strategy. The topology shown represents the consensus of 30 jackknife replicates; nodal support values (JPs) indicate the proportion of replicates in which each clade was recovered. Blue circles mark nodes with maximal support (100). The accompanying bar plot summarizes the frequency of each consensus topology across replicates, exhibiting the two alternative resolutions of early polyneopterans; the Haplocercata-first hypothesis shown here was not recovered by any replicate analysis. (B) Summary of coalescent-based analyses from matrices 1, 2, 5 and 6 (left), alongside ML analyses of matrices 5 and 6 (right). The weighted ASTRAL (wASTRAL) tree is derived from the matrix 1 coalescent analysis, while the ML tree is based on site-homogeneous partitioned model analysis of matrix 5. Supports of focal nodes are visualized using colour blocks. For ML analyses, colour intensity is determined by the lower of the SH-aLRT and UFBoot support values (with values scaled to a 0–1 range to align with wASTRAL support metric). The left columns represent results from matrix 5, while the right columns show results from matrix 6.

both site-homogeneous and site-heterogeneous models with inadequate compositional categories. This result aligns with recent prevailing transcriptome-based phylogenies using compositionally site-homogeneous models [6,9], underscoring the importance of further evaluating infinite mixture models in phylogenetic inference to ensure a complete exploration of the data. In contrast, analyses employing parameter-richer infinite mixture models (e.g. CAT-PMSF and CAT-GTR) consistently place Zoraptera as the earliest-diverging polyneopteran lineage, with Dermaptera and Plecoptera forming the subsequent branch (figures 3 and 4). Moreover, both jackknifing analyses and coalescent analyses under MSC model provide independent validation of this topology (figure 5). As such, our results in this study provide strong support for the Zoraptera-first hypothesis, while the alternative Haplocercata arrangement is best interpreted as a phylogenetic artefact driven by systematic biases in suboptimal models.

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(b) Morphological evidence and its limitations in resolving the 'Zoraptera problem'

How does morphological evidence from extant and fossil insects contribute to the long-debated phylogenetic placement of Zoraptera? Zorapterans possess numerous plesiomorphies, such as the morphology of the pro- and mesothoracic spinae, pleural arm and membranous anapleural suture [39]. A close relationship between Zoraptera and the early-diverging Dermaptera and Plecoptera has been suggested by several morphological studies. Silvestri [17], who described Zoraptera in 1913, discussed superficial similarities between the order and Dermaptera. Both notably share gregarious habits [40] and maternal care in some species [41]. However, the Zoraptera-first hypothesis supported by our analyses suggests that these traits should be interpreted as symplesiomorphies. Besides, Zoraptera, Plecoptera and Dermaptera share the absence of the muscle *Musculus hypopharyngomandibularis*, a feature also present in Palaeoptera [26]. Therefore, this character represents a symplesiomorphy and provides no phylogenetically informative signal for resolving the position of Zoraptera.

Usazoros hubbardi shares its number of male diploid chromosomes ($2n = 38$, with an XY sex-determination system) with the dermapteran *Prolabia* (within Polyneoptera, this condition is otherwise present only in some Isoptera and Phasmatodea) [42,43]. Kuznetsova *et al.* hypothesized an ancestral karyotype of $2n = 40$ for Zoraptera [42], which is shared with the dermapteran species, *Nala lividipes* (belonging to Labiduridae), and within Polyneoptera is otherwise found only in some Isoptera [43]. Contrary to Kuznetsova *et al.* [42], Jankásek *et al.* [44] described the karyotypes of three Zorapteran species ($2n$♂; ♀ = 42; 42 in *Brazilozoros huxleyi*, $2n$♂; ♀ = 44; 43 in *Brazilozoros kukalovae* and $2n$♂; ♀ = 36; 36 in *L. cacaoensis*), and revealed that they possess predominantly monocentric chromosomes and exhibit both X0 and XY sex chromosome systems. These findings highlight the substantial karyotypic variation and the diversity of sex-determination systems within Zoraptera, underscoring the complex patterns of karyotype evolution among Polyneoptera. Notably, Zoraptera and Dermaptera were previously proposed as the only polyneopteran orders possessing holocentric chromosomes (with all other polyneopteran orders exhibiting monocentric chromosomes), a trait that has been interpreted as a potential synapomorphy supporting the monophyly of Haplocercata [9,45]. However, based on current understanding of insect chromosome structure, this condition is apparently a plesiomorphic character, because holocentric chromosomes are also documented in Palaeoptera [9]. Under the Zoraptera-first phylogenetic framework recovered in the present study, the observed distribution of holocentric chromosomes across polyneopteran lineages becomes considerably more plausible.

Friedrich and Beutel found similarities in the thoracic skeletal structures of Zoraptera and Plecoptera, such as the shape of the propleural sclerites (also shared with Blattodea) [39]. Besides, both orders likewise possess reduced ovipositors [46], although given the placement of Zoraptera presented here it is likely that such reductions are independent—a conclusion that is in accordance with the repeated reduction of the ovipositor across Polyneoptera. In the head, Zoraptera and Plecoptera share tormae without mesal extensions and five incisivi on the left and right mandibles [26]. Zorapteran winged morphs have a kidney-shaped frontal spot also present in some plecopterans [47], although the homology of this structure is unclear [23]. Based on the polyneopteran evolutionary framework recovered in the present study, the observed similarities in head and wing structures between Zoraptera and Plecoptera are interpreted as the result of convergent evolution. Kukalová-Peck and Peck [20] observed that the head shape in Zoraptera with antennal articulations close to the ventral cranial margin represents a plesiomorphy within Insecta, similar to fossil pterygotes from the Palaeozoic. This character demonstrates that Zoraptera has retained plesiomorphic traits of early pterygote insects, thereby underscoring its primitive condition. Taken together, morphological evidence from diverse anatomical structures suggests that Zoraptera is an ancient yet highly specialized lineage, a combination that has long obscured its precise phylogenetic placement.

The Dermaptera+Plecoptera clade is supported by wing base structure, namely the morphology of the ventral basisubcostale, and the articulation between the antemedian notal wing process and first axillary sclerite [48]. Besides, both groups share the absence of male gonostyli and a functional ovipositor (features also present in Zoraptera, and thus probably representing plesiomorphic conditions rather than synapomorphies for Dermaptera+Plecoptera) and paired male gonopore [48,49]. An early-diverging Dermaptera+Plecoptera clade (dubbed Dermoplectopterida) [50] was recovered in some ribosomal RNA analysis [51–53] and early phylotranscriptomic studies [5,35], and is now supported by our own analyses accounting for common sources of systematic error.

Reconstructing the ancestral state of the polyneopteran last common ancestor depends critically on resolving phylogenetic relationships among early-diverging orders. Although Zoraptera represents the earliest-diverging lineage, as discussed earlier, it is a lineage that is both ancient and highly specialized. Many zorapteran characters, such as their highly reduced wing venation, probably represent derived adaptations for subcortical life in late-stage decayed wood and consequences of extreme miniaturization, as zorapterans do not exceed 3 mm in length [5,18,23]. Therefore, the ancestral polyneopteran probably differed significantly from zorapterans. Besides, the recovered sister-group relationship between Plecoptera (with aquatic nymphs) and Dermaptera further suggests a terrestrial ancestry for Polyneoptera [9], challenging the prevailing hypothesis of aquatic origins for early flying insects [54,55].

(c) Multiple models corroborate a congruent insect tree of life

Insects, the most species-rich animal group, represent a critical challenge for understanding the origins and evolution of their diversity. While genomic data have revolutionized insect systematics, reshaping traditional views on fleas, lice, termites and twisted-winged insects [6,10,56,57], deep phylogenetic conflicts persist. Key unresolved nodes include the 'Paleoptera problem' (Odonata, Ephemeroptera and Neoptera) and the monophyly of Acercaria (Psocodea, Thysanoptera and Hemiptera)

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Table 1. Topology testing results for competing hypotheses in IQ-TREE.

tree	logL	deltaL	bp-RELL	p-KH	p-SH	p-WKH	p-WSH	c-ELW	p-AU
H1*	−11402221.92	855.95	0 −	0 −	0 −	0 −	0 −	1.24×10^{-168} −	1.23×10^{-5} −
H2*	−11401365.97	0	1 +	1 +	1 +	1 +	1 +	1 +	1 +
H1	−13147870.17	0	0.885 +	0.886 +	1 +	0.886 +	0.886 +	0.885 +	0.881 +
H2	−13148111.84	241.66	0.115 +	0.114 +	0.114 +	0.114 +	0.114 +	0.115 +	0.119 +

Notes: H1 denotes the Haplocercata-first hypothesis while H2 represents the Zoraptera-first hypothesis. Asterisks indicate results from the CAT-PMSF model, whereas unmarked values correspond to the LG+C60+F+G analysis. Plus signs (+) indicate trees included in the 95% confidence set; minus signs (−) indicate significant rejection at the 5% level.

bp-RELL, Bootstrap proportion using the RELL (resampling estimated log-likelihood) method; c-ELW, Expected Likelihood Weight; deltaL, logL difference from the best tree (the one with the highest logL); logL, log-likelihood of the tree; p-AU, p-value of the Approximately Unbiased (AU) test ; p-KH, p-value of the one-sided Kishino-Hasegawa test; p-SH, p-value of the Shimodaira-Hasegawa test; p-WKH, p-value of the weighted Kishino-Hasegawa test; p-WSH, p-value of the weighted Shimodaira-Hasegawa test.

[14,58]. Despite advances in dataset scale and analytical methods, these recalcitrant nodes underscore lingering limitations in substitution modelling [10].

Historically, insect phylogenomics has relied on site-homogeneous partitioned models to mitigate rate heterogeneity among genes, often neglecting compositional heterogeneity among sites (e.g. [6,57,59–63]). However, site-heterogeneous models, particularly CAT-based approaches, better capture the empirical complexity of amino acid substitution patterns [64,65]. These models demonstrate superior robustness against long-branch attraction artefacts [65] and have resolved deep divergences across diverse lineages (e.g. [58,66–69]).

However, the adoption of site-heterogeneous models in insect phylogenetics remains inconsistent. While some recent works highlight the efficacy of site-heterogeneous models [10,58,70–75], others persist with site-homogeneous models (e.g. [61–63,76–78]) or limit their analyses to computationally tractable approximations, such as finite mixture models (e.g. [73,74,79–81]). This may be because the application of high-parameter models like CAT-GTR has a high computational demand and often experiences convergence issues, restricting their application to reduced matrices (e.g., [75,82). This may introduce uncertainty in tree reconstruction. Emerging solutions, such as PMSF-based approximations (C10–60-PMSF, CAT-PMSF) [83,84] and jackknife resampling [85], offer computationally tractable alternatives that balance model accuracy, convergence efficiency and computational cost, with validation across diverse taxa (e.g. [67,86–89]).

Some studies have suggested that the CAT-GTR model is overparameterized or sensitive to missing data [90,91], leading to poorer performance compared with site-homogeneous models (including partitioned model) [92,93]. In fact, when performing a CAT-GTR analysis, the across-site compositional heterogeneity within the dataset is estimated, and the number of site-frequency categories needed to adequately capture this heterogeneity is inferred [64,94]. This demonstrates that, in theory, the CAT-GTR model does not overfit the data, even when applied to compositionally homogeneous datasets [94]. Moreover, some studies indicate that the partitioned model consistently exhibits more inferior performance than site-heterogeneous models based on ML-framework metrics (AIC and BIC values) and approximate indirect comparison [66,95] (figure 4A). Regardless of the controversy, in practice, model performance can vary depending on the dataset and taxonomic scope, and no single approach is universally applicable. Therefore, criticisms of CAT models warrant scrutiny but should not preclude its use. On the contrary, comprehensive testing of available evolutionary models on specific datasets and objective evaluation of their outputs are essential steps towards a more robust reconstruction of the insect Tree of Life.

In this study, through robust model comparison and topology testing, we have reaffirmed that CAT-based models more accurately capture site-specific substitution processes and exhibit superior model fit. These models also yield a novel topological hypothesis for the phylogenetic position of Zoraptera. Moreover, coalescent-based analyses were used to mitigate potential biases introduced by incomplete lineage sorting, further supporting the Zoraptera-first hypothesis. As a methodological case study, this work will provide a template for future phylogenomic studies in insect Tree of Life.

5. Concluding remarks

The phylogenetic relationships among early-diverging polyneopteran orders—one of the three major clades of Neoptera (wing-folding insects)— remain one of the most persistent challenges in insect evolutionary studies. By analysing the most species-rich and balanced datasets of protein-coding genes that are currently available, we systematically evaluated common sources of tree reconstruction artefacts to elucidate the early evolution of Polyneoptera. Our analyses reveal a robust novel topology, with the enigmatic Zoraptera emerging as the sister group to all other polyneopteran orders, and Dermaptera+Plecoptera (Dermoplectopterida) forming the subsequent branch, in congruence with some previous molecular and morphological studies. Through comparative experiments using diverse models of molecular evolution, we demonstrate that previously proposed polyneopteran relationships are only supported under constrained analytical conditions. Our results underscore the significance of both proper modelling and the integration of both molecular and morphological evidence for resolving contentious issues in insect phylogeny.

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Ethics. This work did not require ethical approval from a human subject or animal welfare committee.

Data accessibility. Detailed information regarding the software used to carry out the phylogenetic analyses, along with specific analytical procedures and associated parameters, is provided in the online Electronic Supplementary Material. Other supporting files of phylogenetic analysis have been uploaded to Figshare [96].

Declaration of AI use. We have not used AI-assisted technologies in creating this article.

Authors' contributions. Y.W.: data curation, formal analysis, investigation, methodology, software, validation, visualization, writing—original draft, writing—review and editing; E.T.: formal analysis, investigation, resources, software, validation, writing—original draft, writing—review and editing; P.K.: data curation, funding acquisition, investigation, resources, validation, writing—original draft, writing—review and editing; M.S.E.: investigation, validation, visualization, writing—original draft, writing—review and editing; J.L.-F: investigation, validation, visualization, writing—original draft, writing—review and editing; Z.-W.Y.: investigation, validation, visualization, writing—original draft, writing—review and editing; O.R.-S.: investigation, validation, visualization, writing—original draft, writing—review and editing; D.H.: investigation, validation, visualization, writing—original draft, writing—review and editing; D.P.: investigation, validation, visualization, writing—original draft, writing—review and editing; P.C.J.D.: funding acquisition, investigation, validation, visualization, writing—original draft, writing—review and editing; C.C.: conceptualization, formal analysis, funding acquisition, methodology, resources, supervision, validation, visualization, writing—original draft, writing—review and editing.

All authors gave final approval for publication and agreed to be held accountable for the work performed therein.

Conflict of interest declaration. We declare we have no competing interests.

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