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Assumptions, Discordance, and Reproducibility Challenges in Species Delimitation During the Phylogenomic Transformation of Insect Systematics

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ABSTRACT

Phylogenomics has transformed insect systematics by expanding the number and genomic distribution of characters available for species delimitation, yet greater data volume does not remove the assumptions that connect sampled organisms to taxonomic conclusions. This methodological review examines that inferential chain across sampling design, locus and genome representation, species-tree models, gene flow, delimitation algorithms, threshold and prior sensitivity, and reproducibility. The central problem is that different analytical layers answer different questions: a mitochondrial partition, genomic cluster, gene tree, multispecies-coalescent result, or morphometric discontinuity is evidence about biological structure, but none is automatically identical to a species boundary. Insect case studies further show that discordance may reflect sex-biased dispersal, hybridization, ancestral introgression, rapid lineage splitting, inadequate taxon sampling, technical contamination, or method-specific partition behaviour. The review therefore treats discordance as information to diagnose rather than noise to suppress. It develops a proposed assumption-to-conclusion architecture in which evidence is carried forward with explicit representation, model, sensitivity, and validation boundaries. The intended outcome is not a universal delimitation rule, but a more reproducible way to state what a species hypothesis is supported by, what assumptions it inherits, where competing interpretations remain plausible, and what additional evidence would discriminate among them.

Keywords: Species delimitation, Phylogenomics, Multispecies coalescent, Gene-tree discordance, Integrative taxonomy, Reproducibility

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Introduction

The phylogenomic transformation of insect systematics has changed the scale of species-delimitation evidence more rapidly than it has changed the logic by which evidence becomes a taxonomic conclusion. Large-scale integrative taxonomy demonstrates why this distinction matters: high-throughput sequence clustering can generate preliminary hypotheses efficiently, but targeted validation is still needed before those hypotheses carry the evidential weight expected of species-level decisions [1]. Species can therefore be treated operationally as

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provisional hypotheses whose credibility depends on congruence among relevant properties rather than as direct readouts of a single analytical layer [2].

In insects, this problem is not abstract. Integrative work on tropical tephritid complexes has shown that combining morphology, genetics, and other biological evidence can either reveal overlooked diversity or collapse nominal taxa when apparently diagnostic differences are not independently sustained [3]. Conversely, DNA barcodes can be highly useful for discovery and triage while remaining insufficient, on their own, as the entire evidential basis for a formal species description [4]. The broader literature on cryptic insect diversity likewise indicates that estimates of hidden species richness are conditioned by the taxa sampled, the evidence brought to bear, and the delimitation methods used [5]. Increased genomic scale therefore cannot be equated automatically with increased taxonomic certainty.

This review treats that mismatch as an inferential chain: sampling determines represented variation; loci record only parts of lineage history; gene trees are modelled as species histories; lineage sorting and gene flow generate discordance; algorithms create partitions; and thresholds, priors, and reporting choices affect stability. Its proposed contribution is an assumption-to-conclusion architecture that keeps these layers separate while asking what each evidence class can support, which alternatives remain open, and what validation is needed before a partition becomes a taxonomic conclusion.

Review scope and method classes

The review focuses on 2017–2023 methodological and insect-systematics literature that directly informs species delimitation, spanning single-locus delimiters, coalescent models, phylogenomic marker strategies, and integrative case studies. These classes estimate different objects. Molecular delimiter performance varies across speciation scenarios, insect-family results change with dataset composition and sampling, and ASAP formalizes choices for ranking distance partitions [6–8]. The relevant comparison is therefore not which program returns the "best" species count, but which assumptions connect its statistical object to the biological hypothesis.

Single-locus tree-process approaches make the same point from a different direction. Multi-rate PTP relaxes the assumption that all delimited species share one intraspecific branching rate, but it still depends on the quality and interpretation of a single-locus genealogy [9]. A method can thus be free of one fixed similarity threshold without being free of biological or statistical assumptions. Software such as SPdel improves transparency by placing several single-locus methods into a common comparative workflow [10], yet concordance among programs should remain an analytical result rather than a surrogate for taxonomic truth.

The proposed method-class distinction used here separates four operations that are often conflated: evidence generation, partitioning rule, genealogical or demographic modelling, and independent validation. Agreement is most informative when it joins evidence classes with substantially different failure modes. Re-running the same locus through several delimiters can characterize algorithmic sensitivity, but it does not create the biological independence supplied by nuclear genomic, morphological, ecological, behavioural, or reproductive evidence.

Sampling assumptions

Sampling determines the variation an analysis can see. Missing geography, sexes, rare lineages, or contact zones can make within-species heterogeneity resemble interspecific discontinuity, or the reverse. A chafer analysis that removed geographic sampling bias still produced oversplitting [11]. Geography therefore remains important without explaining every mismatch between molecular clusters and species hypotheses.

Biological asymmetry can make sampling interact with marker choice. In *Pachypus* beetles, sex-biased dispersal obscured species boundaries because cytoplasmic and nuclear signals responded differently to the movement regime [12]. Mitonuclear discordance therefore cannot be assigned automatically to introgression: asymmetric dispersal can produce a related evidential pattern. The selected sampling studies do not provide an equivalent direct test of life-stage-restricted sampling, so life-stage effects should remain an explicit evidence gap rather than being inferred from adult or mixed-stage data.

Taxon sampling operates at a second scale. Adding rare Blattidae and Anaplectidae improved Blattoidea resolution [13], showing that omitted lineages can change the phylogenetic context of downstream inference without proving that broader sampling always resolves species. In the *Temnothorax salvini* group, ultraconserved elements, mitochondrial genomes, and morphometrics jointly revised an earlier two-species concept into multiple supported lineages [14]. The lesson is not that genomic clusters override morphology, but that independent evidence can test whether a partition remains coherent across data classes.

Sampling assumptions should therefore be reported as properties of the inference, not merely as logistical details. Geographic breadth, representation of potentially dispersal-asymmetric sexes, inclusion of rare or geographically intermediate lineages, and availability of independent phenotypic evidence determine which alternative explanations remain testable. A species hypothesis supported under restricted sampling may remain useful, but its scope should not exceed the variation actually observed.

Locus and genome representation

Moving from a barcode to hundreds or thousands of loci changes more than precision: it changes sampled genomic histories, orthology decisions, missingness, and which processes are averaged or retained. Insect phylogenomics reviews show that target-capture, transcriptomic, and whole-genome strategies differ in coverage and analytical trade-offs [15]. “Genome scale” is therefore not one evidential state; dense marker panels, low-coverage genomes, and mitochondrial barcodes represent different portions of lineage history.

One response has been to standardize nuclear markers so that datasets become more comparable and reusable across taxa [16]. Empirical benchmarking of universal single-copy orthologues showed that, in the tested animal lineages, standardized nuclear markers distinguished closely related morphospecies more consistently than classical mitochondrial barcodes [17]. The boundary is important: this supports the value of nuclear representation in those lineages, not a universal claim that morphology is correct or that one nuclear panel will delimit all insects reliably.

Low-coverage whole-genome sequencing illustrates a complementary trade-off. It can recover large phylogenomic marker sets from comparatively shallow sequencing, expanding the genomic substrate available for inference [18]. Yet marker recovery is conditional on sequencing depth, assembly strategy, and downstream orthology decisions, so successful phylogenetic reconstruction cannot itself validate species boundaries. Technical provenance is equally important. In Cicadidae, cross-sample contamination altered phylogenomic signal sufficiently to require explicit detection and removal [19]. Apparent gene-tree conflict can therefore be biological or technical, and those alternatives must be separated before discordance is interpreted.

Representation can consequently fail in at least three different senses: the chosen locus may capture an atypical lineage history; genome-scale sampling may still represent genomic regions unevenly; or technical processing may corrupt the relationship between specimen and sequence. These failures should not be pooled under the vague heading “data quality,” because each requires a different corrective test.

Because representation choices change what a delimitation result can mean, a prose inventory of marker technologies is insufficient. **Table 1** separates representation states by the biological history sampled, the failure mode they expose, and the additional test required before species-level interpretation.

Table 1. Representation states that change the taxonomic meaning of phylogenomic evidence

Locus or genome representation state			
Evidential standing	**Taxonomic context · Evidence**	**Interpretive consequence · Limits and uncertainty**	**Research or test**
Mitochondrial single-locus representation			
Evidence-derived	Recently diverged insect lineages; barcode-led discovery One non-recombining maternal genealogy with broad cross-dataset comparability	Efficient hypothesis generation but vulnerable to discordance with nuclear or phenotypic structure Sex-biased dispersal, introgression, lineage sorting, and locus-specific history can affect the partition	Proposed — test candidate boundaries with independent nuclear and organismal evidence
Standardized nuclear-marker representation			
Evidence-derived	Cross-study delimitation and comparative taxonomy Homologous nuclear loci designed for reuse across lineages	Improves comparability and reduces dependence on one mitochondrial history Standardization does not remove sampling, gene-flow, or model assumptions	Proposed — benchmark against independently delimited taxa and discordant cases
Broad single-copy orthologue panels			
Evidence-derived	Closely related lineages with genome-scale sampling Distributed nuclear loci with explicit orthology	Can improve congruence with independently recognized morphospecies in tested lineages Performance remains lineage-dependent and conditional on orthology and sampling	Proposed — replicate across divergent insect orders and difficult recent radiations

	Low-coverage whole-genome representation		
Evidence-derived	Phylogenomic datasets built from shallow sequencing Large marker sets recovered from low-depth genomic reads	Expands genomic coverage without guaranteeing species-boundary accuracy Assembly, depth, missingness, and recovery filters shape represented history	Proposed — test delimitation stability across plausible assembly and locus-selection alternatives
	Contaminated or provenance-uncertain representation		
Evidence-derived	Multi-sample phylogenomics Loci or samples with anomalous phylogenetic placement or identity conflict	Can generate false discordance that resembles biological history True introgression or lineage sorting can coexist with technical error	Proposed — require sample- and locus-level QC before biological interpretation
	Representation audit before delimitation		
Proposed synthesis	Any genome-scale insect dataset Explicit record of marker type, genome fraction, assembly, orthology, missingness, and QC	Separates “more data” from “better representation” The audit itself cannot validate a species boundary	Use the audit to identify which competing histories remain testable

Species-tree assumptions

Species-tree methods do not simply convert gene trees into a more authoritative history. Multispecies-coalescent delimitation can fail under model violations, detect population structure rather than taxonomic species, and over-split some geographically widespread lineages [20–22]. This does not invalidate the multispecies coalescent (MSC); it means its output inherits assumptions about population structure, isolation, sampling, and the process generating gene-tree variation.

The distinction becomes sharper in phylogenomics because high locus counts can increase support while historical identifiability remains poor. Aphididae phylogenomics recovered thousands of orthologues yet still encountered deep discordance associated with short branches, introgression, and anomaly-zone behaviour [23]. Strong support for one summary tree therefore does not imply that competing histories have disappeared. For delimitation, the relevant question is whether observed discordance is expected under the fitted lineage model, whether another process could generate a similar pattern, and whether independent evidence justifies interpreting the recovered structure as a species boundary.

This distinction creates an interpretation boundary between three statements that should not be collapsed: genes contain structured genealogical signal; a species-tree model explains part of that signal; and a taxonomic species hypothesis is sufficiently supported. The first two can be statistically strong while the third remains conditional. Conversely, discordance does not automatically invalidate the species hypothesis when it is an expected consequence of recent divergence.

The species-tree problem is therefore best visualized as parallel inferential lanes rather than one route from loci to species. **Figure 1** identifies where genealogical support, model fit, discordance diagnosis, and taxonomic interpretation must remain separate.

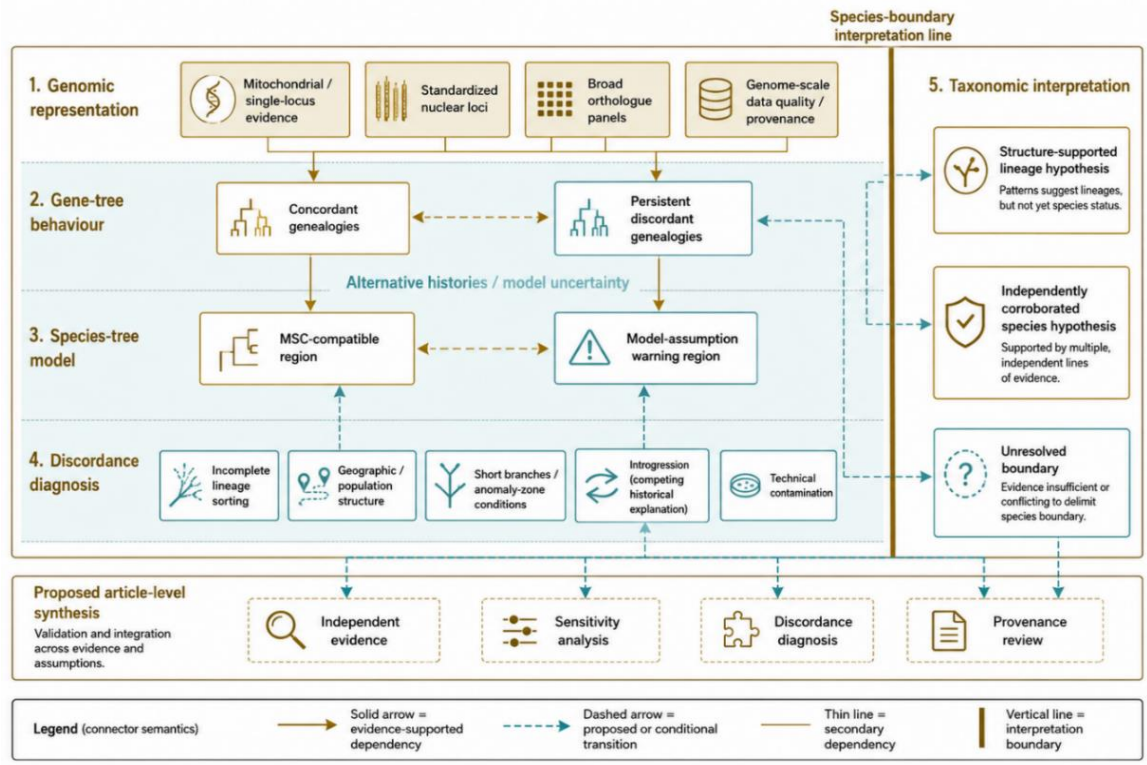


Figure 1. The inference boundary between gene-tree concordance, species-tree fit, and taxonomic species status

Gene flow and discordance

Discordance is informative only when its possible generators remain distinct. Incomplete lineage sorting is part of the MSC's null genealogical process, whereas migration and introgression require explicit extensions or alternative models; cross-species gene flow can alter species-tree estimation and delimitation [24–26]. Treating conflict as noise discards demographic information, but treating every conflict as introgression exceeds what discordance alone can identify.

Taxonomic interpretation is equally conditional. Species delimitation with gene flow does not yield a universal migration cutoff between species and populations [27]. Gene-flow estimates are demographic parameters, not taxonomic verdicts; their meaning depends on timing, genomic distribution, lineage structure, and independent evidence. A low estimated exchange rate does not itself demonstrate complete reproductive isolation, just as detectable exchange does not by itself collapse two supported lineage hypotheses into one species.

Odonate phylogenomics provides a deep-time example: ancestral introgression contributed substantially to conflict among genomic histories [28]. By contrast, North American *Culicoides* provide a more recent boundary problem in which genomic clusters, mitochondrial haplotypes, and hybridization evidence do not align perfectly [29]. These situations should not be collapsed into one category of "gene flow." Ancient introgression may alter reconstruction of lineage history without implying present-day reproductive compatibility, whereas contemporary hybridization can complicate assignments among lineages even when genomic differentiation remains detectable.

Discordance can also reverse the direction of taxonomic revision. In the *Bactrocera frauenfeldi* complex, nuclear phylogenomics combined with morphology and biogeography supported synonymy and showed that COI did not reliably separate most focal taxa [30]. Genome scale therefore did not simply reveal more species; it changed the interpretation of earlier partitions. Discordance is most useful when it identifies which assumption or evidence class may be failing, not when averaged into a consensus score.

The proposed synthesis therefore distinguishes generative discordance from interpretive discordance. Generative discordance concerns why genomic histories disagree—lineage sorting, gene flow, introgression, dispersal asymmetry, or technical artefact. Interpretive discordance concerns disagreement among evidence classes or algorithms about the taxonomic partition. The distinction matters because additional loci may help characterize the first while leaving the second unresolved. A defensible species hypothesis should state which form of discordance is present, which explanations were actually tested, and which remain plausible.

Because the same observed conflict can arise at different biological and analytical scales, **Table 2** separates discordance states by generative process, evidential signature, interpretation boundary, and the further test needed before species-level inference.

Table 2. Discordance states that require different biological explanations before species-level interpretation

Discordance state		
Evidence · Interpretive consequence	**Research or test · Biological setting**	**Evidential standing · Uncertainty or competing explanation**
Incomplete lineage sorting during rapid divergence		
Gene trees disagree while variation remains compatible with coalescent lineage sorting **Conflict can be expected without requiring reticulation**	Proposed — compare lineage-sorting and migration-aware explanations and inspect branch-length conditions Recently separated lineages or short internodes	Evidence-derived Gene flow or model misspecification can produce overlapping patterns
Population structure interpreted as species structure		
Strong genomic partitioning supported within a fitted coalescent model **Statistical support can exceed the taxonomic information content of the partition**	Proposed — expand geographic sampling and test whether independent evidence tracks the partition Geographically widespread or internally structured taxa	Evidence-derived Restricted sampling or continuous geographic structure can mimic discrete species boundaries
Recent gene flow or hybridization		
Nuclear admixture, hybrid genotypes, or mitochondrial–nuclear assignment mismatch **No-gene-flow models may distort lineage or species-tree inference**	Proposed — use migration-aware models and independent organismal evidence Contacting or incompletely isolated insect lineages	Evidence-derived Sex-biased dispersal and ancestral polymorphism can contribute to similar discordance
Ancient introgression		
Genomic regions retain conflicting historical relationships beyond simple technical noise **A single bifurcating tree may incompletely represent lineage history**	Proposed — test reticulation explicitly and avoid inferring current reproductive compatibility Deep insect radiations with reticulate history	Evidence-derived Incomplete lineage sorting and short branches can produce overlapping conflict
Sex-biased dispersal with organellar–nuclear mismatch		
Mitochondrial structure differs from nuclear structure in a manner consistent with dispersal asymmetry **A mitochondrial partition may exaggerate or obscure a species boundary**	Proposed — combine nuclear loci, sex-specific natural-history information, and geographic sampling Taxa with asymmetric male and female movement	Evidence-derived Introgression remains a competing explanation unless independently evaluated
Technical discordance		
Anomalous locus or sample placement consistent with contamination or provenance failure **Biological interpretation is premature until data integrity is checked**	Proposed — audit sample identity, locus provenance, assembly, and contamination before demographic interpretation Large multi-sample phylogenomic matrices	Evidence-derived Biological discordance can coexist with technical error
Evidence-class reversal of a prior partition		
Integrative evidence supports lumping or splitting differently from an earlier single-class hypothesis **Discordance can justify revision in either direction rather than systematically increasing species number**	Require the conclusion to identify which evidence conflicts, which is independently corroborated, and which remains unresolved Complexes in which morphology or COI conflicts with nuclear phylogenomics	Proposed synthesis No evidence class is an infallible ground truth

Delimitation algorithms

Algorithmic disagreement is itself evidence about inferential sensitivity. In Sri Lankan Sericini, multiple COI-based delimiters both split and lump morphospecies, with limited agreement among methods [31]. The appropriate response is therefore not to elect a species partition by majority vote. Methods should instead be compared

according to the biological or statistical process they model: scenario-dependent performance differs among delimiters [6], tree-process methods retain genealogy-specific assumptions [9], and comparison pipelines preserve value only when individual outputs and settings remain visible [10].

The proposed distinction is between partition agreement and evidential corroboration. Agreement among algorithms applied to the same locus measures analytical stability; corroboration requires information with partly independent failure modes. A stable single-locus partition may therefore remain a discovery hypothesis rather than a taxonomic conclusion.

Threshold and prior sensitivity

Increasing data volume can sharpen a model-dependent result without resolving whether the model's taxonomic interpretation is appropriate. Analyses of Bayesian species delimitation show that increasing locus number can strengthen support for population splits under particular multispecies-coalescent formulations [32]. Greater posterior confidence should therefore not be confused with independent validation of species status.

Threshold dependence creates a parallel problem. Sequence-similarity thresholds used for molecular operational units vary with marker and dataset properties [33]. Although such metabarcoding thresholds are not formal species criteria, they demonstrate why fixed cut-offs should not be transferred uncritically across datasets.

The proposed sensitivity principle is that every taxonomic partition should be interpreted against a decision neighbourhood: the set of plausible priors, thresholds, models, and sampling representations under which the conclusion either persists or changes. **Table 3** organizes the principal failure modes, while **Figure 2** separates numerical stability from taxonomic robustness.

Table 3. Conditional states in which numerical stability can overstate species-boundary robustness

Evidential signal · Interpretive consequence	Failure or boundary condition · Required test	Evidential standing	Analytical context
Locus-count reinforcement			
Increasing support as loci accumulate **Statistical confidence can increase without independent species validation**	Population structure may satisfy the fitted model Reassess biological corroboration as information increases	Evidence-derived	Coalescent delimitation
Prior-sensitive partition			
Species assignments change across defensible prior settings **Result is conditional on model specification**	Poorly informed demographic priors can dominate weakly identified parameters Report prior sensitivity explicitly	Proposed synthesis	Bayesian delimitation
Similarity-threshold instability			
Cluster composition changes across plausible cut-offs **Operational units are threshold-dependent**	Marker and dataset composition alter optimal similarity criteria Evaluate multiple defensible thresholds without equating MOTUs with species	Evidence-derived	Molecular clustering
Algorithm-stable but evidence-poor partition			
Several methods recover similar boundaries **Agreement demonstrates analytical stability, not independent corroboration**	Shared locus history or shared assumptions can generate correlated agreement Add evidence with different failure modes	Proposed synthesis	Repeated analyses of one evidence class
Sensitivity-transparent species hypothesis			
Partition persists across plausible analytical alternatives and independent evidence **Taxonomic interpretation becomes better supported, but not infallible**	Unsampled geography, gene flow, or untested biological traits can remain State residual alternatives and validation needs	Proposed synthesis	Integrative delimitation

The distinction between a stable estimate and a robust taxonomic inference requires a visual separation of parameter space, partition behaviour, and biological validation.

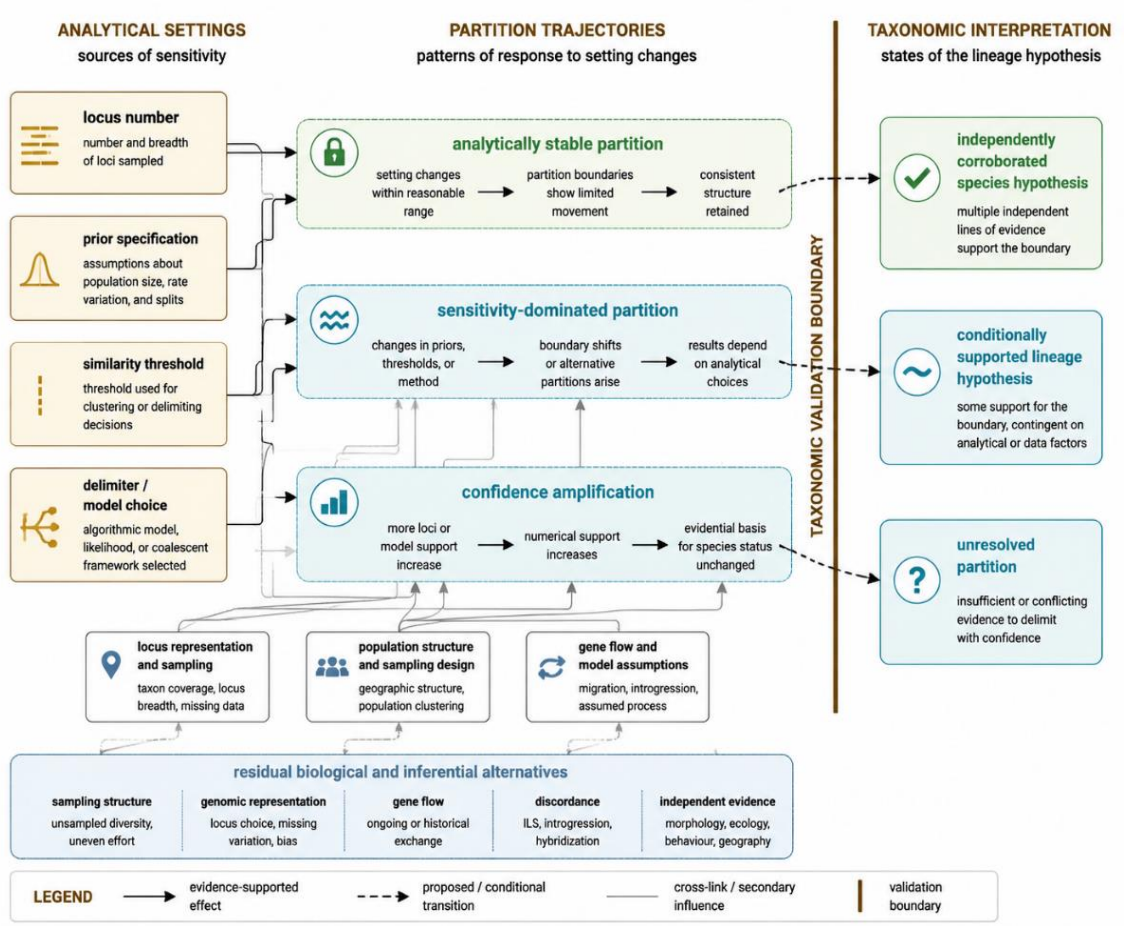


Figure 2. Decision neighbourhoods separating analytical stability from taxonomic robustness

Reproducibility and data availability

Reproducibility begins before code deposition. Biodiversity records increasingly lose direct linkage to specimens, weakening the ability to reassess identifications and associated evidence [34]. Phylogenomic reproducibility additionally requires sufficient provenance to detect technical failures such as cross-sample contamination [19]. Rapid molecular taxonomy can likewise become difficult to reassess when comparative diagnoses, source information, or other critical data are omitted [35].

The practical implication is that reproducibility must connect specimen, sequence, assembly, locus filtering, software version, parameterization, and final taxonomic interpretation. Depositing sequences without preserving those links permits re-use but may not permit reconstruction of the inference.

Reporting standard

This review therefore proposes an assumption-to-conclusion reporting standard. Preliminary clustering and later validation should be distinguished explicitly [1]. Technical quality control should be documented before biological discordance is interpreted [19]. Barcode evidence should be identified as one evidence class rather than presented automatically as a complete species diagnosis [4], while algorithmic disagreement should remain visible rather than being reduced to consensus [31].

At minimum, reports should identify the sampling frame, represented genomic fraction, species-tree assumptions, treatment of gene flow, delimiter and software settings, prior or threshold sensitivity, independent corroborating evidence, unresolved discordance, and specimen-to-sequence provenance. Comparative software can facilitate reproducibility when settings remain recoverable [10]; specimen linkage supplies an independent provenance requirement [34]; and critical descriptive or comparative information should not disappear in the pursuit of analytical speed [35].

This standard is proposed rather than prospectively validated. Its purpose is narrower: to make explicit which inferential steps support a species hypothesis and which remain conditional.

Conclusion

Phylogenomics expands the evidential resolution available to insect systematics but does not eliminate the assumptions connecting sampled organisms to species hypotheses. Sampling, genomic representation, species-tree models, gene flow, delimiter choice, thresholds, priors, and provenance can each alter interpretation. Discordance should therefore be diagnosed rather than suppressed, and analytical confidence should remain distinct from taxonomic corroboration. The proposed reporting architecture does not define species universally; it makes the inferential path to a species hypothesis more explicit, reproducible, and open to reassessment.

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References

1. Hartop E, Srivathsan A, Ronquist F, Meier R. Towards large-scale integrative taxonomy (LIT): Resolving the data conundrum for dark taxa. Syst Biol. 2022;71(6):1404-22. doi:10.1093/sysbio/syac033
2. Wells T, Carruthers T, Muñoz-Rodríguez P, Sumadijaya A, Wood JRI, Scotland RW. Species as a heuristic: Reconciling theory and practice. Syst Biol. 2022;71(5):1233-43. doi:10.1093/sysbio/syab087
3. Schutze MK, Virgilio M, Norrbom A, Clarke AR. Tephritid integrative taxonomy: Where we are now, with a focus on the resolution of three tropical fruit fly species complexes. Annu Rev Entomol. 2017;62:147-64. doi:10.1146/annurev-ento-031616-035518
4. Zamani A, Fric ZF, Gante HF, Hopkins T, Orfinger AB, Scherz MD, et al. DNA barcodes on their own are not enough to describe a species. Syst Entomol. 2022;47(3):385-9. doi:10.1111/syen.12538
5. Li X, Wiens JJ. Estimating global biodiversity: The role of cryptic insect species. Syst Biol. 2023;72(2):391-403. doi:10.1093/sysbio/syac069
6. Luo A, Ling C, Ho SYW, Zhu CD. Comparison of methods for molecular species delimitation across a range of speciation scenarios. Syst Biol. 2018;67(5):830-46. doi:10.1093/sysbio/syy011
7. Magoga G, Fontaneto D, Montagna M. Factors affecting the efficiency of molecular species delimitation in a species-rich insect family. Mol Ecol Resour. 2021;21(5):1475-89. doi:10.1111/1755-0998.13352
8. Puillandre N, Brouillet S, Achaz G. ASAP: Assemble species by automatic partitioning. Mol Ecol Resour. 2021;21(2):609-20. doi:10.1111/1755-0998.13281
9. Kapli P, Lutteropp S, Zhang J, Kobert K, Pavlidis P, Stamatakis A, et al. Multi-rate Poisson tree processes for single-locus species delimitation under maximum likelihood and Markov chain Monte Carlo. Bioinformatics. 2017;33(11):1630-8. doi:10.1093/bioinformatics/btx025
10. Ramirez JL, Valdivia P, Rosas-Puchuri U, Valdivia NL. SPdel: A pipeline to compare and visualize species delimitation methods for single-locus datasets. Mol Ecol Resour. 2023;23(8):1959-65. doi:10.1111/1755-0998.13864
11. Lukic D, Eberle J, Thormann J, Holzschuh C, Ahrens D. Excluding spatial sampling bias does not eliminate oversplitting in DNA-based species delimitation analyses. Ecol Evol. 2021;11(15):10327-37. doi:10.1002/ece3.7836
12. Eberle J, Bazzato E, Fabrizi S, Rossini M, Colomba M, Cillo D, et al. Sex-biased dispersal obscures species boundaries in integrative species delimitation approaches. Syst Biol. 2019;68(3):441-59. doi:10.1093/sysbio/syy072
13. Deng W, Luo X, Ho SYW, Liao S, Wang Z, Che Y. Inclusion of rare taxa from Blattidae and Anaplectidae improves phylogenetic resolution in the cockroach superfamily Blattoidea. Syst Entomol. 2023;48(1):23-39. doi:10.1111/syen.12560
14. Prebus MM. Phylogenomic species delimitation in the ants of the Temnothorax salvini group (Hymenoptera: Formicidae): An integrative approach. Syst Entomol. 2021;46(2):307-26. doi:10.1111/syen.12463

15. Johnson KP. Putting the genome in insect phylogenomics. Curr Opin Insect Sci. 2019;36:111-7. doi:10.1016/j.cois.2019.08.002
16. Eberle J, Ahrens D, Mayer C, Niehuis O, Misof B. A plea for standardized nuclear markers in metazoan DNA taxonomy. Trends Ecol Evol. 2020;35(4):336-45. doi:10.1016/j.tree.2019.12.003
17. Dietz L, Eberle J, Mayer C, Kukowka S, Bohacz C, Baur H, et al. Standardized nuclear markers improve and homogenize species delimitation in Metazoa. Methods Ecol Evol. 2023;14(2):543-55. doi:10.1111/2041-210X.14041
18. Zhang F, Ding Y, Zhu CD, Zhou X, Orr MC, Scheu S, et al. Phylogenomics from low-coverage whole-genome sequencing. Methods Ecol Evol. 2019;10(4):507-17. doi:10.1111/2041-210X.13145
19. Owen CL, Marshall DC, Wade EJ, Meister R, Goemans G, Kunte K, et al. Detecting and removing sample contamination in phylogenomic data: An example and its implications for Cicadidae phylogeny (Insecta: Hemiptera). Syst Biol. 2022;71(6):1504-23. doi:10.1093/sysbio/syac043
20. Barley AJ, Brown JM, Thomson RC. Impact of model violations on the inference of species boundaries under the multispecies coalescent. Syst Biol. 2018;67(2):269-84. doi:10.1093/sysbio/syx073
21. Sukumaran J, Knowles LL. Multispecies coalescent delimits structure, not species. Proc Natl Acad Sci U S A. 2017;114(7):1607-12. doi:10.1073/pnas.1607921114
22. Chambers EA, Hillis DM. The multispecies coalescent over-splits species in the case of geographically widespread taxa. Syst Biol. 2020;69(1):184-93. doi:10.1093/sysbio/syz042
23. Owen CL, Miller GL. Phylogenomics of the Aphididae: Deep relationships between subfamilies clouded by gene tree discordance, introgression and the gene tree anomaly zone. Syst Entomol. 2022;47(3):470-86. doi:10.1111/syen.12542
24. Jiao X, Flouri T, Yang Z. Multispecies coalescent and its applications to infer species phylogenies and cross-species gene flow. Natl Sci Rev. 2021;8(12):nwab127. doi:10.1093/nsr/nwab127
25. Jackson ND, Carstens BC, Morales AE, O'Meara BC. Species delimitation with gene flow. Syst Biol. 2017;66(5):799-812. doi:10.1093/sysbio/syw117
26. Jiao X, Flouri T, Rannala B, Yang Z. The impact of cross-species gene flow on species tree estimation. Syst Biol. 2020;69(5):830-47. doi:10.1093/sysbio/syaa001
27. Jiao X, Yang Z. Defining species when there is gene flow. Syst Biol. 2021;70(1):108-19. doi:10.1093/sysbio/syaa052
28. Suvorov A, Scornavacca C, Fujimoto MS, Bodily P, Clement M, Crandall KA, et al. Deep ancestral introgression shapes evolutionary history of dragonflies and damselflies. Syst Biol. 2022;71(3):526-46. doi:10.1093/sysbio/syab063
29. Shults P, Hopken M, Eyer PA, Blumenfeld A, Mateos M, Cohnstaedt LW, et al. Species delimitation and mitonuclear discordance within a species complex of biting midges. Sci Rep. 2022;12:1730. doi:10.1038/s41598-022-05856-x
30. Doorenweerd C, San Jose M, Geib S, Dupuis J, Leblanc L, Barr N, et al. A phylogenomic approach to species delimitation in the mango fruit fly (Bactrocera frauenfeldi) complex: A new synonym of an important pest species with variable morphotypes (Diptera: Tephritidae). Syst Entomol. 2023;48(1):10-22. doi:10.1111/syen.12559
31. Ranasinghe UGSL, Eberle J, Thormann J, Bohacz C, Benjamin SP, Ahrens D. Multiple species delimitation approaches with COI barcodes poorly fit each other and morphospecies: An integrative taxonomy case of Sri Lankan Sericini chafers (Coleoptera: Scarabaeidae). Ecol Evol. 2022;12(5):e8942. doi:10.1002/ece3.8942
32. Leaché AD, Zhu T, Rannala B, Yang Z. The spectre of too many species. Syst Biol. 2019;68(1):168-81. doi:10.1093/sysbio/syy051
33. Bonin A, Guerrieri A, Ficetola GF. Optimal sequence similarity thresholds for clustering of molecular operational taxonomic units in DNA metabarcoding studies. Mol Ecol Resour. 2023;23(2):368-81. doi:10.1111/1755-0998.13709
34. Troudet J, Vignes-Lebbe R, Grandcolas P, Legendre F. The increasing disconnection of primary biodiversity data from specimens: How does it happen and how to handle it? Syst Biol. 2018;67(6):1110-9. doi:10.1093/sysbio/syy044
35. Zamani A, Vahtera V, Sääksjärvi IE, Scherz MD. The omission of critical data in the pursuit of 'revolutionary' methods to accelerate the description of species. Syst Entomol. 2021;46(1):1-4. doi:10.1111/syen.12444