Eurasia Specialized Veterinary Publication

Entomological Research Letters

ISSN: 3108-639X

2021, Volume 1, Issue 2, Page No: 30-40
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Available online at: www.esvpub.com/

Integrative Insect Species Delimitation Requires Explicit Reconciliation of Morphological Evidence, Molecular Signals, and Biological Meaning

Youssef Hariri[1]*, Hassan Nasser[1], Fatima Zahra[2]

[1]Department of Integrative Taxonomy and Species Delimitation, Faculty of Sciences, Mohammed V University, Rabat, Morocco.
[2]Department of Systematic Entomology and Molecular Systematics, Faculty of Biosciences, University of Casablanca, Casablanca, Morocco.

***E-mail** ✉ youssef.hariri@um5.ac.ma

ABSTRACT

Species delimitation in insects increasingly combines morphology, DNA barcodes, multilocus phylogenies, genomic partitions, geography, ecology and reproductive information. Yet integration is often treated as accumulation: concordant signals are counted as support, whereas discordant signals are framed as problems to be resolved by choosing a preferred dataset. This conceptual analysis argues that the principal difficulty is instead inferential. Different evidence classes record different biological processes and operate at different temporal, spatial and organismal scales; consequently, a molecular cluster, morphological diagnosis or ecological association cannot be converted directly into a species conclusion without an intervening interpretation step. Drawing on recent insect-systematics and delimitation literature, the article distinguishes observation from process inference and taxonomic decision, evaluates the complementary limits of morphological, molecular, ecological and reproductive evidence, and identifies recurrent causes of discordance including population structure, model misspecification, introgression, hybridization, symbiont effects, sex-biased dispersal, geographic history and technical error. The article then develops a proposed reconciliation architecture in which evidence is evaluated for biological meaning, independence, boundary conditions and alternative explanations before a taxonomic state is assigned. The approach does not prescribe universal weights, diagnostic thresholds or a single species concept. Its intended contribution is a transparent logic for deciding when concordant evidence is genuinely reinforcing, when discordance is biologically informative, and when unresolved cases should remain open to reassessment.

Keywords: Integrative taxonomy, Insect systematics, Species delimitation, DNA barcoding, Morphology, Mitonuclear discordance.

Received: 20 September 2021
Revised: 08 November 2021
Accepted: 12 November 2021

How to Cite This Article: Hariri Y, Nasser H, Zahra F. Integrative Insect Species Delimitation Requires Explicit Reconciliation of Morphological Evidence, Molecular Signals, and Biological Meaning. Entomol Res Lett. 2021;1(2):30-40.
https://doi.org/10.51847/vWRwOTIRMd

Introduction

Species delimitation is not merely the act of partitioning specimens into named units. In insect systematics it links organismal variation, lineage history and nomenclatural decisions, and its errors propagate into ecological inference, biodiversity estimates and comparative biology. Integrative work on tephritid fruit flies has shown that combining evidence can both reveal cryptic diversity and collapse unsupported nominal distinctions, demonstrating that "integration" does not inherently imply more splitting [1]. The same point appears in disparate insect systems: ambush bugs require reconciliation of molecular, morphometric and ecological information, butterfly classifications can change when classical taxonomy is compared with multispecies-coalescent inference, and cryptic harvester ants can remain unresolved by any single dataset [2–4].

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Genome-scale evidence has increased the number of characters available for species delimitation, but not eliminated the interpretive problem. In the *Temnothorax salvini* group, ultraconserved elements, mitochondrial data, morphometrics and geography jointly discriminated competing species hypotheses, illustrating the gain from integration rather than genomic substitution for organismal evidence [5]. Equally important, taxonomic claims remain reproducible only when specimens, identification procedures and taxon concepts are traceable; a survey of entomological practice found substantial deficiencies in such validation reporting [6].

The problem addressed here is therefore narrower than whether integrative taxonomy is useful. It is how heterogeneous evidence should be reconciled when each class can reflect different processes. Morphological difference may diagnose a lineage without identifying the history that produced it; mitochondrial divergence may track geography or introgression; genomic structure may resolve population subdivision more precisely than species independence; and reproductive or ecological differences may be informative without being complete. This article develops a proposed reasoning architecture that inserts an explicit interpretive layer between observed signal and taxonomic conclusion. It does not seek a universal numerical score or replace existing species concepts. Instead, it asks what each signal can establish, what alternative processes can generate it, and what additional evidence would be required before moving from candidate partition to defensible species delimitation.

Materials and Methods

This conceptual analysis organizes the selected literature by evidential function rather than by technological sophistication. Four classes are treated as analytically distinct: morphological evidence, molecular evidence, ecological and reproductive evidence, and contextual evidence governing interpretation. The latter includes geography, demographic history, sampling design, model assumptions and data quality. Geography is not merely metadata: differentiation maintained in sympatry has a different implication from divergence that increases continuously with geographic distance, and explicit spatial tests can help distinguish species-level separation from isolation by distance [7].

The first reconciliation rule is that a statistical partition is not itself a species statement. Multispecies-coalescent methods can delimit structured populations even when the generating process is population subdivision rather than speciation [8]. Gene flow further complicates this translation because divergence can proceed under migration, making strict-isolation assumptions inappropriate in some histories [9]. Model adequacy therefore matters: plausible violations of coalescent assumptions can change inferred boundaries, so fit must be evaluated rather than assumed [10].

The second rule is that algorithmic agreement should not be treated as a vote independent of model conditions. Simulations show that molecular delimitation methods behave differently across combinations of effective population size, divergence and gene flow [11], while single-locus approaches differ in their tendencies toward over-splitting and over-lumping [12]. ASAP is consequently best interpreted as a rapid way to generate ranked candidate partitions rather than a taxonomic endpoint [13]. In a large Chrysomelidae benchmark, sampling, geographic coverage, taxonomic difficulty and method choice all affected molecular delimitation performance [14]. Spatially widespread taxa create an additional risk because structured populations can be over-split under coalescent models [15]. Accordingly, three known problems—population structure, model violation and broad geographic structure—can all produce partitions that require biological interpretation rather than automatic taxonomic elevation [8, 10, 15].

The proposed logic used throughout this article therefore separates three stages: observation, process interpretation and taxonomic inference. Evidence is considered stronger when independent classes support the same lineage hypothesis for biologically compatible reasons, not simply when multiple analyses return the same partition. Conversely, discordance triggers examination of alternative explanations before one dataset is privileged. This is a conceptual rule for organizing evidence, not an empirically calibrated weighting system.

Results and Discussion

Morphology remains indispensable because species are ultimately described and diagnosed through organismal characters, yet its evidential meaning depends on what was sampled. Life stage is a major boundary: next-generation barcoding has enabled adult–larva matching in Odonata, showing how molecular association can recover morphological information from otherwise disconnected stages without itself proving species

independence [16]. Sex can matter similarly. In a recent grasshopper radiation, genomic and phenotypic evidence produced delimitation inferences sensitive to the character set and to sex-specific variation [17].

Morphological characters also differ in diagnostic value within the same taxonomic problem. In *Choristoneura*, voucher-based comparisons showed that wing colour and genitalic differences did not contribute equally to discrimination, and overlap prevented a simple rule in which one visual character alone settled species status [18]. Conversely, morphology should not be assumed to be a low-resolution relic that genomics must replace. Genome-scale data in the *Melanoplus scudderi* complex supported species previously diagnosed morphologically despite problematic limited-marker phylogenies [19].

The relevant distinction is therefore between morphological diagnosis and evolutionary interpretation. *Berberomeloe* beetles show that qualitative morphology, morphometrics and mitochondrial structure can be broadly concordant even when morphometric spaces overlap [20]. In *Lionepha* ground beetles, genome skimming gained taxonomic meaning only when interpreted with classical morphology, type material and nomenclatural history [21]. Morphology can thus provide direct diagnosability, reveal life-stage or sex-specific structure, and anchor names to specimens, but it does not by itself identify whether observed differences arose through reproductive isolation, geography, plasticity or other processes. Its contribution is strongest when the measured characters, life stages and taxonomic anchors are explicit.

Molecular evidence

Molecular evidence encompasses several non-equivalent signals. Tree-based delimiters such as multi-rate Poisson tree processes infer candidate partitions from branching patterns and model assumptions; they do not directly test biological independence [22]. Multilocus studies can broaden the evidential basis, but locus histories may still disagree. In Afrotropical *Culicoides*, mitochondrial and nuclear loci broadly supported several relationships while one nuclear locus produced discordant placements and missing-data limitations remained relevant [23]. Multilocus analysis of *Phoebis* butterflies similarly illustrates how molecular data can adjudicate competing taxonomic hypotheses without establishing a rule that molecular evidence categorically outranks morphology [24].

The most important boundary is between mitochondrial pattern and genome-wide lineage history. In *Melitaea didyma*, deep COI structure comprised more mitochondrial lineages than principal genome-wide ddRAD groupings; probable introgression and *Wolbachia*-associated effects were among the biological explanations considered [25]. *Empria* sawflies provide the converse warning: nuclear genomic data and morphology supported species despite mitochondrial sharing, while both introgression and sequence contamination had to be considered as causes of conflict [26]. Thus, “molecular evidence” should not be treated as a single class whose components necessarily share a genealogy.

Task definition also matters. High-throughput DNA barcoding of the British bee fauna achieved strong correspondence with morphological identifications, yet still produced split and lumped molecular entities and technical artefacts [27]. Such workflows demonstrate the value of barcodes for assignment and monitoring, but identification accuracy does not constitute validation of a species-delimitation rule.

A concise prediction-and-test ledger is useful because the same molecular pattern can imply different biological histories depending on locus type, spatial sampling and model fit. **Table 1** therefore separates observable molecular states from the additional evidence required before taxonomic interpretation.

Table 1. Molecular signal states and the tests needed before converting sequence partitions into species-level inference

Molecular evidence state	Evidence · Permitted interpretation	Research or test	Boundary condition or uncertainty	Biological setting · Evidential standing
Single-locus partition	Discrete sequence clusters or branch-rate shifts Candidate lineage hypothesis	Test additional loci, geography and organismal evidence	Genealogy may reflect population structure, stochastic lineage sorting or method behaviour	Barcode or gene-tree discovery Evidence-supported use; reconciliation rule proposed
Concordant multilocus partition	Recurrent grouping across loci Stronger support for lineage continuity	Examine model fit and external evidence classes	Shared demographic history and model misspecification can still align loci	Several independently sampled loci Proposed interpretation rule

Deep mitochondrial split with weak genomic separation	Strong COI divergence but limited nuclear/genomic differentiation Mitochondrial history requires separate explanation	Test nuclear structure, gene flow, geography and symbionts	Introgression, symbiont association and geographic structure may contribute	Geographically structured or introgressing populations Evidence-supported pattern; proposed decision use
Mitochondrial sharing with nuclear/morphological separation	Shared or weakly differentiated mtDNA; distinct nuclear/organismal evidence mtDNA is insufficient to reject the species hypothesis	Replicate sequencing, inspect vouchers and expand nuclear evidence	Introgression, lineage sorting or technical contamination remain alternatives	Recently diverged or introgressing taxa Evidence-supported pattern; proposed decision use
Genome-wide population structure	Strong genomic clustering Evidence of restricted gene exchange or historical structure	Evaluate spatial continuity, migration and biological differentiation	Population structure need not equal species-level independence	Widespread or fragmented taxa Evidence-supported concern; proposed decision use
Reference-barcode assignment	High specimen-to-reference correspondence Reliable identification within the reference framework	Keep assignment performance separate from delimitation validity	Reference error, NUMTs, contamination, cryptic diversity and taxon incompleteness	Monitoring or identification workflow Evidence-supported distinction

Ecological and reproductive evidence

Ecological and reproductive information supplies biological meaning that sequence divergence alone cannot provide, but these data are also context dependent. Sex-biased dispersal can alter marker interpretation because maternally inherited loci may record a different spatial history from the nuclear genome. In *Pachypus* beetles, strong sex-biased dispersal obscured species boundaries and promoted misleading mitochondrial structure [28]. This is evidence for a mechanism that can create discordance, not a general reason to discount mitochondrial data across insects.

Reproductive evidence is similarly informative without functioning as a universal binary criterion. Comparative work in *Drosophila* found substantial but incomplete correspondence between coalescent species boundaries and reproductive isolation [29]. The mismatch matters because lineage divergence can precede complete isolation, while some differentiated populations may continue exchanging genes. Explicit models of species divergence with gene flow show that migration changes the expected relationship between within- and between-lineage genetic similarity [30]. Ecological association, dispersal and reproductive isolation should therefore be treated as independent biological evidence about how populations interact, rather than as automatic confirmation or rejection of a molecular partition.

Sources of discordance

Discordance is not a single condition. It is the observable consequence of multiple biological, historical and technical processes, and its interpretation should begin by asking which alternatives are compatible with the taxon and sampling design. Hybridization and reproductive mode are especially consequential in insects with complex life histories. In the balsam woolly adelgid complex, integrative evidence revised a six-name taxonomy toward two species while identifying hybrids and effects of clonality and invasion history [31]. Apparent morphological or genetic intermediacy therefore cannot be assigned to species status without considering hybrid origin and reproductive system.

Geography can also produce correlated phenotypic and genetic structure that resembles species differentiation. In *Xylotrupes siamensis*, post-refugial history and isolation by distance explained substantial genetic and phenotypic variation without requiring multiple species [32]. Yet geographic sampling bias is not an all-purpose explanation for molecular over-splitting. In a single-locality sample of Sericini scarabs, DNA-based methods still showed substantial mismatch with morphospecies, indicating that method behaviour and biological history can remain important after large-scale spatial structure is reduced [33].

Finally, discordance may occur within an otherwise coherent integrative result. In *Dicerapanorpa*, morphology, geometric morphometrics, barcodes, phylogeny and several delimitation algorithms were broadly concordant, but not identical; method-specific disagreements remained even where the overall taxonomic interpretation was strong [34]. Likewise, mitochondrial patterns can conflict with nuclear and organismal evidence for distinct reasons, including introgression, contamination and sex-biased dispersal [25, 26, 28]. Discordance should therefore trigger causal alternatives, not an automatic search for the dataset to be discarded.

Because these mechanisms are easily conflated in prose, **Table 2** distinguishes discordance states by the process capable of generating them and by the test that would meaningfully reduce uncertainty.

Table 2. Discordance states that require different taxonomic responses rather than a single rule for conflicting evidence

Discordance state		
Evidential standing · Limits and uncertainty	**Evidence · Interpretive consequence**	**Discriminating test or evidence need · Biological setting**
Geographic structure without clear secondary-contact persistence		
Evidence-supported process; proposed response **Refugial history and isolation by distance can mimic lineage boundaries**	Genetic and/or phenotypic differentiation increases with distance Do not equate spatial partition with species independence	Denser geographic sampling; tests of sympatric or contact-zone persistence Broadly distributed populations
Mitonuclear discordance		
Evidence-supported alternatives; proposed response **Introgression, lineage sorting, symbionts or dispersal asymmetry may differ among taxa**	mtDNA and nuclear/genomic partitions disagree Treat marker histories separately	Nuclear replication, migration analysis, symbiont screening and spatial context Introgressing, sex-biased or recently diverged taxa
Hybrid or clonal lineage ambiguity		
Evidence-supported process; proposed response **Reproductive mode and recent hybridization can obscure ancestry**	Intermediate or conflicting phenotype/genotype assignments Named forms may not represent independent species	Hybrid-assignment models, reproductive data and replicated genomic evidence Hybridizing or cyclically parthenogenetic taxa
Algorithmic over-splitting		
Evidence-supported concern; proposed response **Spatial control alone may not remove the problem**	More molecular entities than organismally supported units Molecular partitions remain hypotheses	Compare models, loci, sampling schemes and independent evidence Single-locus or MSC-based delimitation
Technical discordance		
Evidence-supported concern **Contamination, NUMTs, reference errors or sample mix-ups can mimic gene flow**	Unexpected marker sharing or outlier placements Biological interpretation should be deferred	Resequencing, voucher reinspection and independent loci Sequence-based workflows
Partial multi-evidence disagreement		
Evidence-supported pattern; proposed response **No majority vote identifies the true generating process**	Most evidence classes agree but one method or character conflicts Retain conflict explicitly rather than force unanimity	Target the discrepant mechanism or measurement directly Otherwise integrative datasets

Evidential reconciliation architecture

The preceding sections demonstrate that morphological, molecular, ecological and reproductive evidence classes do not fail because one class is inherently superior to another. They fail when a signal generated at one biological level is interpreted as if it directly represented another. A mitochondrial lineage is a history of mitochondrial inheritance; a genomic cluster is a pattern of allele distribution; a morphological difference is an observed phenotype; and reproductive evidence describes particular biological interactions under particular conditions. The central problem is therefore not evidence scarcity but inference transfer.

This article proposes an evidential reconciliation architecture in which species delimitation proceeds through three interpretive questions. First, what was directly observed? Second, which biological processes could plausibly generate that observation? Third, does the resulting interpretation support movement toward a taxonomic decision? This separation is designed to prevent molecular partitions, morphological diagnoses or ecological associations from being treated as interchangeable evidence units.

The architecture does not assign fixed weights to evidence classes. Such weighting would imply that one evidence type has a universal meaning across insects, despite strong differences among taxa, life stages, dispersal systems, reproductive modes and evolutionary histories. Instead, it emphasizes conditional interpretation. A mitochondrial split in a geographically structured butterfly, for example, requires different reasoning from a multilocus genomic pattern supported by morphology and geography. Likewise, morphological overlap in a recently diverged beetle complex cannot be interpreted identically to overlap caused by phenotypic plasticity or incomplete sampling.

A central feature of the proposed architecture is an explicit discordance pathway. When evidence classes disagree, the initial response is not selection of a preferred dataset but identification of alternative explanations. These may include incomplete lineage sorting, introgression, hybridization, population structure, model violation, sampling limitations, technical artefacts or genuine biological complexity. The appropriate next analysis depends on which explanation is plausible for the system under study.

This logic is supported by recurring empirical observations across insects. Integrative studies of ants, butterflies, beetles, flies and other groups frequently resolve competing hypotheses by combining evidence rather than by replacing one class with another [3-5, 19, 34]. However, the exact combination that is informative differs among systems. Therefore, the proposed architecture treats evidence classes as complementary sources of biological information rather than as interchangeable measurements of a single hidden variable.

Figure 1 summarizes this proposed reconciliation logic by separating evidence generation, interpretation boundaries, discordance routes and taxonomic decisions.

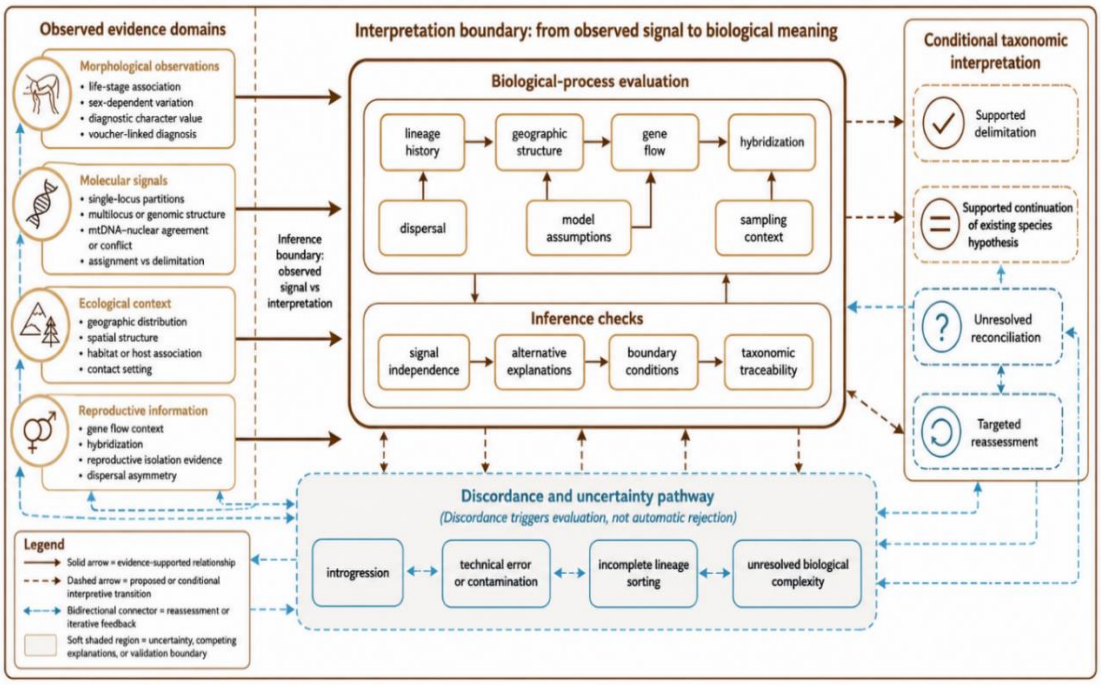


Figure 1. Biological meaning must be inferred before evidence classes are converted into insect species-delimitation decisions

Decision states for species delimitation

Species delimitation decisions are often represented as binary outcomes: species or not species. However, the evidence reviewed here indicates that many difficult insect complexes occupy intermediate interpretive conditions in which evidence is informative but not yet sufficient for a final taxonomic decision. A binary structure can therefore hide uncertainty rather than resolve it.

This article proposes four conceptual decision states: supported delimitation, supported continuation of an existing species hypothesis, unresolved reconciliation, and targeted reassessment. These states are not confidence scores, statistical categories or validated prediction classes. They are an organizational framework for describing how evidence should influence reasoning.

A supported delimitation state occurs when independent evidence classes converge in a biologically interpretable way. The strongest cases are not those with the largest number of datasets, but those where different evidence classes provide compatible information about lineage history. Integrative analyses of *Temnothorax*, *Melanoplus*, *Berberomeloe* and *Dicerapanorpa* demonstrate examples in which morphology, molecular evidence and geographic or ecological information jointly contributed to interpretation [5, 19, 20, 34].

A supported continuation state is equally important because integration may prevent unnecessary splitting. Deep mitochondrial divergence, geographic structuring or algorithmic partitions may appear taxonomically compelling while representing population history rather than species independence. Studies of widespread taxa, scarab beetles and mitochondrial discordance demonstrate why molecular entities should not automatically become named species [15, 25, 33].

The unresolved reconciliation state is not a failure of analysis. It is a scientifically meaningful outcome when available evidence classes describe different histories and current data cannot discriminate among plausible explanations. For example, mitochondrial sharing may result from introgression, incomplete lineage sorting or technical problems; intermediate morphology may reflect hybridization, polymorphism or incomplete sampling. Forcing a binary conclusion in such situations may produce less defensible taxonomy than maintaining an explicit unresolved state [26, 31].

The targeted reassessment state identifies what additional evidence would most efficiently reduce uncertainty. The relevant evidence depends on the discordance mechanism. Nuclear genomic data may resolve mitochondrial conflict, geographic sampling may clarify isolation-by-distance patterns, reproductive data may test biological differentiation, and voucher review may reveal technical problems. This state-based approach does not replace taxonomic judgement; it makes the basis of judgement more transparent.

Table 3 provides the proposed decision-state register and separates evidence-supported observations from the article's proposed organizational framework.

Table 3. Decision states for separating evidence interpretation from final species-delimitation action

Evidence · Interpretive consequence	**Limits and uncertainty · Biological setting**	**Research or test · Evidential standing**
Supported delimitation		
Concordant morphology, molecular patterns, geography or reproductive evidence **Candidate species boundary has strong biological support**	Degree of support remains taxon dependent Multiple independent evidence classes converge	Continue diagnostic and nomenclatural evaluation Proposed synthesis based on evidence patterns
Supported continuation of existing species hypothesis		
Population structure, barcode divergence or algorithmic clusters without broader support **Avoid unnecessary splitting**	Hidden diversity may remain unresolved Molecular or ecological partition conflicts with organismal evidence	Add independent evidence before revision Proposed synthesis based on documented risks
Unresolved reconciliation		
Discordant mitochondrial, nuclear, morphological or ecological patterns **Maintain uncertainty rather than force a decision**	Alternative explanations remain plausible Evidence classes imply different histories	Target mechanism-specific testing Proposed synthesis
Targeted reassessment		
Evidence points toward sampling, model, demographic or technical limitations **Additional evidence can address the limiting factor**	New evidence may support or reject current hypotheses Specific cause of disagreement identified	Select tests according to the suspected process Proposed synthesis

Integrative species delimitation has progressed from combining multiple datasets toward understanding why those datasets disagree. The distinction is important because evidence accumulation alone does not solve inference

problems. More data may increase confidence in a biological interpretation, but only if the additional data measure processes relevant to the taxonomic question.

The literature reviewed here supports three broad conclusions. First, no evidence class is universally primary. Morphology can diagnose species and anchor names, but it may be incomplete across life stages, sexes or recent radiations. Molecular data can reveal hidden structure, but partitions depend on inheritance patterns, demographic history, sampling and analytical assumptions. Ecological and reproductive evidence can provide biological context, but their interpretation depends on the system examined.

Second, discordance should be considered information rather than noise. Mitochondrial and nuclear disagreement has revealed introgression, symbiont effects, dispersal differences and technical issues in insect studies [25, 26, 28]. Geographic structure has explained some apparent species differences without requiring additional taxa, while other cases show that controlling spatial sampling does not eliminate all molecular over-splitting [32, 33]. The correct response to disagreement is therefore explanation, not selection.

Third, species delimitation requires explicit separation between evidence and taxonomic action. A molecular entity may be a useful discovery unit without being an accepted species. A morphological diagnosis may be valuable without revealing the evolutionary process behind it. A reproductive difference may indicate biological divergence without representing a complete isolation barrier. Maintaining these distinctions allows taxonomic decisions to remain transparent.

The proposed reconciliation architecture should therefore be viewed as a framework for reasoning rather than a replacement for existing delimitation methods. It does not provide universal thresholds, predictive accuracy estimates or a single species criterion. Its purpose is to reduce category errors: confusing identification with delimitation, genetic structure with species history, diagnosis with mechanism and disagreement with analytical failure.

Future evaluation of this framework would require application across insect groups differing in dispersal, reproductive systems, life histories, geographic ranges and evolutionary ages. Particularly valuable tests would compare cases with independent external knowledge of species boundaries and examine whether explicit reconciliation changes the transparency or reproducibility of taxonomic decisions. Such validation remains a future requirement rather than an established outcome.

Consequences for taxonomic practice

The practical consequence of explicit reconciliation is not slower taxonomy but more transparent taxonomy. Rapid molecular workflows have clear value for specimen assignment, biodiversity assessment and discovery, yet assignment and delimitation represent different analytical tasks [27]. Treating every molecular partition as a species hypothesis without additional interpretation risks converting methodological output into taxonomic conclusion.

Taxonomic practice also depends on preserving links between names, specimens and evidence. Reviews of entomological reporting have highlighted weaknesses in identification validation, while taxonomic revisions demonstrate that genomic information gains meaning when connected to classical characters and type concepts [6, 21]. The objective is not to preserve morphology as the sole authority but to ensure that every evidence class is interpreted within a reproducible taxonomic framework.

The increasing availability of automated delimitation methods should therefore be understood as expanding hypothesis generation rather than removing the need for taxonomic reasoning. Tree-based and distance-based approaches can identify candidate boundaries, but the transition from candidate to species requires evaluation of biological meaning, alternative explanations and nomenclatural consequences [13, 22].

Finally, accelerated species description should not omit comparative information required to distinguish genuinely independent taxa from structured populations, geographic variants or methodological artefacts. Critical discussions of rapid taxonomy have emphasized that new methods should not replace the comparative evidence needed for reliable species recognition [35].

Conclusion

Integrative insect species delimitation is strongest when evidence classes are reconciled according to the biological processes they represent rather than simply combined by accumulation. Morphological, molecular, ecological and

reproductive information can each provide valuable evidence, but none should be interpreted outside its biological and methodological boundaries.

The central contribution of this article is a proposed reconciliation logic that separates observation, process interpretation and taxonomic decision. It treats discordance as a source of biological information, preserves uncertainty when evidence remains ambiguous, and encourages targeted evidence collection rather than automatic splitting or lumping. Species delimitation is therefore not improved by choosing a single superior dataset, but by making explicit why each dataset supports, limits or challenges a particular biological interpretation.

Acknowledgments: None

Conflict of Interest: None

Financial Support: None

Ethics Statement: None

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