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Gene Flow, Analytical Assumptions, and Taxonomic Consequences in Contemporary Insect Species Delimitation

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ABSTRACT

Species delimitation in insects increasingly relies on genomic data, yet greater molecular resolution does not automatically resolve the distinction between population structure and species boundaries. The central difficulty is inferential: ongoing or historical gene flow, uneven geographic and life-stage sampling, model misspecification, prior choices, and discordance among mitochondrial, nuclear, morphological, ecological, and reproductive evidence can all change the partitions recovered by analytical methods. This critical review evaluates how those sources of uncertainty alter taxonomic interpretation rather than treating delimiter output as a species diagnosis. Evidence from recent insect systems shows that gene flow may coexist with persistent genomic differentiation, that mitochondrial partitions may either exaggerate or obscure lineage boundaries, and that geographic structure or locus number can alter inferred species counts. The review therefore advances a proposed validation architecture in which species hypotheses are stress-tested across sampling, demographic, model, and prior perturbations before taxonomic action. The key distinction is between detecting structured evolutionary lineages and justifying species rank. Robust delimitation should consequently emphasize evidence independence, explicit competing explanations, sensitivity analysis, and transparent retention of uncertainty when data cannot discriminate among introgression, incomplete lineage sorting, population structure, or genuine species divergence.

Keywords: Species delimitation, Gene flow, Multispecies coalescent, Introgression, Population structure, Integrative taxonomy.

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Introduction

Genomic datasets have changed the scale at which insect lineages can be detected, but they have not removed the conceptual problem of deciding when lineage structure warrants species rank. Recent syntheses distinguish discovery of genetic partitions from validation of those partitions as species hypotheses [1, 2]. Barcode-based partitions remain useful for identifying candidate diversity, yet mitochondrial clustering alone does not provide an independent demonstration of a species boundary [3]. This distinction is especially important in insects, where population subdivision, geographic isolation, host association, hybridization, and marker-specific histories can each generate discrete molecular patterns.

The problem is not that structured populations are biologically unimportant. It is that a delimitation model can assign strong support to structure without establishing the taxonomic interpretation of that structure. Under the multispecies coalescent (MSC), simulated population subdivisions can be delimited even when the generative

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scenario does not define them as separate species [4]. Accordingly, posterior support, bootstrap support, barcode gaps, or clustering stability should be treated as properties of an analytical result rather than as taxonomic verdicts. This review evaluates contemporary insect species delimitation through four linked inferential boundaries: biological permeability versus species persistence, sampling representation versus apparent lineage structure, model fit versus numerical support, and evidential discordance versus taxonomic action. The article's principal contribution is a proposed validation logic in which candidate species are evaluated across these boundaries before nomenclatural decisions are made. The proposal does not establish a universal species criterion; it organizes where confidence should be gained, qualified, or withheld.

Species delimitation under ongoing gene flow

Strict-isolation models are most vulnerable when divergence has occurred with continuing migration or episodic hybridization. Modeling gene flow explicitly can alter delimitation outcomes relative to isolation-only formulations [5]. Migration-aware extensions of the MSC provide a useful sensitivity test because they ask whether a proposed boundary persists when exchange among populations is admitted, although their conclusions remain conditional on parameter identifiability and on how migration and divergence are modeled [6]. Gene flow itself therefore cannot be used as a simple lumping rule. Theoretical work shows that migration can alter the relationship between within- and between-population divergence, and long-term migration need not generate the same genomic signature as episodic introgression [7].

Insect contact zones illustrate why permeability and species status must remain analytically separate. In Phyciodes butterflies from western Canada, genome-wide differentiation persisted among closely related species despite occasional hybridization and low gene flow, while mitochondrial COI could be shared [8]. Thus, mitochondrial sharing is compatible with continued genomic differentiation in at least some contact systems, but the result cannot be generalized into a universal tolerance for hybridization.

Conversely, mitochondrial diagnostics can also fail by assigning misleading boundaries. Genome-scale work in a Bactrocera pest complex showed that mtDNA barcode diagnoses did not consistently contain the species limits indicated by genomic data [9]. At deeper phylogenetic scales, whole-genome analysis of Leptocircini butterflies implicated ancient gene flow as one plausible contributor to discordance among systematic signals [10]. These cases establish a constrained conclusion: gene flow can produce or preserve discordance, but discordance alone does not identify gene flow, nor does detected exchange determine whether populations should be named separately.

The proposed interpretive rule is therefore conditional. A species hypothesis under ongoing gene flow should survive at least two questions: whether differentiation persists after a biologically plausible migration model is admitted, and whether independent evidence indicates that the partition is more than geography or marker history. Failure of either question should increase uncertainty rather than automatically force splitting or lumping.

Sampling effects

Sampling is part of the inferential model because it determines which geographic, genomic, morphological, and life-history variation is visible. In allopatric Lepidoptera, genomic differentiation across regions and continents was heterogeneous and did not map simply onto mitochondrial divergence [11]. Such structure can reflect long-term separation, isolation by distance, or incipient divergence, but allopatry removes direct observation of contemporary reproductive contact. A geographically narrow sample can therefore understate continuity, whereas sparse sampling across a broad range can turn continuous structure into apparently discrete clusters.

The effect is measurable in large molecular datasets. Across a species-rich chrysomelid dataset, molecular-delimitation efficiency varied with features including haplotype representation, geographic sampling, and taxonomic difficulty [12]. These associations do not produce a universal minimum sample size, but they reject the idea that sampling is neutral preprocessing.

Representation also operates through biology. Adult-centered collecting can omit immature forms and associations that matter for arthropod systematics [13]. Broad geographic sampling of Asian cavity-nesting honeybees revealed differentiated groups that required taxonomic interpretation rather than automatic naming [14]. In Monochamus sawyer beetles, coalescent boundary evaluation also depended on how biallelic data and phasing were handled [15]. Nylanderia ants demonstrate a different sampling axis: integration of ultraconserved elements with male genital morphometrics supported some boundaries while failing to corroborate all previously

reported COI clades [16]. Because male material is not equally available, evidential strength is partly constrained by specimen composition.

These studies support a proposed multidimensional concept of sampling sufficiency. Geographic coverage, number and genomic distribution of loci, representation of life stages or sexes, and availability of independent phenotypic or ecological characters should be evaluated separately. Increasing one dimension cannot be assumed to compensate for deficiency in another. Sampling adequacy is therefore not a numerical threshold but a claim-specific question: was the dataset capable of revealing the alternative explanation most likely to challenge the proposed species boundary?

Model assumptions

More data can strengthen an answer to the wrong model. Simulation and methodological studies show that MSC delimitation can become confidently misleading when population structure, gene flow, or other departures from model assumptions are interpreted as species divergence [17–19]. The central problem is not Bayesian inference itself, but the conversion of model support into species status without testing whether the generative assumptions are biologically plausible.

Geography is a particularly important source of violation. In geographically widespread taxa, structured populations can be over-split by MSC analyses when within-species subdivision is represented as multiple terminal lineages [20]. This risk must be distinguished from the opposite possibility that genuinely distinct cryptic species also show geographic structure. The relevant test is therefore not whether structure exists, but which demographic history can account for it.

Alternative delimiters do not remove this dependency. SODA uses multi-locus quartet frequencies to provide scalable species delimitation, yet its partitions remain genealogical inferences whose taxonomic meaning requires external validation [21]. Models that incorporate an explicit speciation process attempt to distinguish population lineages from species lineages more directly [22], but they introduce their own process assumptions. Machine-learning approaches expand the range of possible classifiers, while remaining dependent on the demographic scenarios, features, and labels represented during training and validation [23]. CLADES similarly demonstrates how classification can be learned from population-genetic summaries under specified scenarios, without making classifier output equivalent to taxonomic truth [24].

The article therefore proposes a support × adequacy distinction. Numerical support asks whether the data favor a partition conditional on a model. Adequacy asks whether the model, sampling frame, and comparison set are capable of representing plausible alternatives. Strong support with weak adequacy should not be treated as strong taxonomic evidence.

The recurring inferential states are easier to compare side by side than in sequential prose; **Table 1** separates the assumption being made from the evidence that can challenge it and from the taxonomic consequence of leaving it untested.

Table 1. Model-assumption failures that can convert lineage structure into unstable species inference

Consequence for species within its limit	Assumption state with evidential standing	Biological setting with observed basis	Discriminating check
Isolation-based support may overstate independence **Exchange may be ongoing, asymmetric, episodic, or ancient**	Isolation-only history Evidence-supported concern	Diverging populations with plausible migration or hybridization Genomic differentiation accompanied by evidence compatible with exchange	Reanalyse under a biologically plausible migration model
Population partitions may be converted into species hypotheses **True cryptic species can also be geographically structured**	Population structure treated as species divergence Evidence-supported concern	Broadly distributed or allopatric lineages Strong geographic clustering without independent reproductive validation	Compare structure, isolation-by-distance, and species-level alternatives
A well-supported lineage may be mistaken for a taxonomic endpoint **Gene-tree accuracy and species status are different questions**	Genealogical partition treated as taxonomic validation Proposed adjudication rule	Multi-locus tree or quartet-based delimiters Stable gene-tree or quartet partitions	Require independent biological or taxonomic corroboration

The method detects lineage separation but not necessarily the process defining species **Speciation process is rarely observed directly**	Speciation process omitted Proposed adjudication rule	Recently diverged populations Coalescent support under a lineage model without an explicit transition to species status	Treat process-aware models as sensitivity analyses, not automatic arbiters
Apparent accuracy may not transfer to unrepresented histories **Domain shift, feature dependence, and label assumptions**	Training scenario treated as empirical domain Evidence-supported concern	Machine-learning delimiters High classifier performance under represented simulation classes	Test performance under alternative demographic scenarios
Agreement may be overinterpreted as independent corroboration **Shared model structure can create pseudo-concordance**	Cross-method agreement treated as independence Proposed adjudication rule	Multiple delimiters sharing data or assumptions Concordant partitions from analytically related methods	Audit dependence among methods before counting concordance

Prior sensitivity

Prior sensitivity is a separate inferential problem because posterior species support combines the data with assumptions about population sizes, divergence times, assignments, and sometimes topology. In Chanohirata leafhoppers, molecular species delimitation was evaluated under multiple BPP prior schemes, making the stability of conclusions across priors directly inspectable [25]. The lesson is not that a particular prior is preferable for insects, but that a single unreported prior configuration can conceal analytical fragility.

Uncertainty may also enter before the prior is specified. Work on nymphalid butterflies showed that uncertainty in topology and initial taxonomic assignments can be propagated rather than fixed as if known [26]. This matters because a guide tree or starting partition can constrain which species histories are evaluated. Whole-genome analysis of the Papilio machaon complex further illustrates that species-level divergence and within-species geographic differentiation can coexist within one radiation, with different taxonomic implications across lineages [27].

The proposed minimum practice is therefore to separate three questions: whether posterior support is stable across defensible priors, whether topology and population assignments have been treated as uncertain where appropriate, and whether conclusions persist when demographic exchange is admitted. Robustness across these perturbations does not prove species status. Instability, however, identifies where taxonomic confidence should be reduced and where independent evidence becomes more important.

Introgression versus population structure

Mito-nuclear discordance is an observation, not a mechanism. In Aeschyntelus bugs, mitochondrial and nuclear datasets produced discordant histories within an integrative taxonomic analysis [28]. Such discordance can be consistent with introgression, but incomplete lineage sorting, geographic structure, sex-biased processes, marker-specific effective population size, or analytical error can produce overlapping patterns. The taxonomic task is therefore to compare explanations rather than attach a mechanism to the discordance itself.

Strong population structure creates a complementary problem. In the Bactrocera frauenfeldi complex, phylogenomic and population-structure evidence revealed pronounced geographic differentiation, while integrative interpretation supported synonymy rather than accepting every automated partition as a species [29]. The structure was biologically real; the error risk lay in converting it directly into rank.

Integrative phylogenomics can also move decisions in opposite directions within the same complex. In Acromyrmex ants, combined phylogenomic, population-genetic, morphological, and biogeographic evidence helped distinguish a supported separate lineage from geographically variable entities interpreted within a broader species-level system [30]. In Stylops, the number of species inferred by SODA increased strongly with the number of loci, whereas the integrative taxonomic interpretation supported both synonymy and undescribed diversity [31]. Method instability therefore does not diagnose false taxonomy, but it is a reason to test whether the recovered units remain coherent under alternative analytical resolutions.

The proposed distinction is between a reticulation hypothesis and a structure hypothesis. Reticulation predicts exchange across lineages that otherwise retain evidence of differentiation; structure predicts subdivision that may arise within a broader species without requiring interspecific exchange. Because both can produce clustered genomes and discordant markers, evidence should be organized around what would discriminate them, not around which explanation is more intuitively attractive.

Table 2 is needed because the same observable pattern can imply different taxonomic actions depending on which alternative explanation remains viable. The ledger therefore separates the observed state from the response needed before interpreting it as introgression, population structure, or a species boundary.

Table 2. Discordance states that require different responses before introgression or population structure is translated into species rank

Evidential basis	Taxonomic context	Immediate interpretive consequence, competing explanation or boundary, and required response before taxonomic act
Mito-nuclear discordance		
Evidence-supported distinction		
Mitochondrial grouping conflicts with nuclear or genome-scale relationships	Recently or historically diverged insect lineages	Marker histories cannot be collapsed into one species history Introgression, incomplete lineage sorting, structure, marker-specific history, or error remain possible Test competing demographic histories and seek independent evidence
Genome-wide differentiation with localized exchange		
Evidence-supported distinction		
Persistent nuclear differentiation alongside evidence compatible with hybridization	Contact-zone species	Gene flow need not erase lineage integrity Amount, timing, and direction of exchange may be poorly identified Evaluate whether differentiation persists under migration-aware models
Strong geography-matched genomic clusters		
Evidence-supported distinction		
Population structure aligns closely with spatial separation	Island, continental, or allopatric populations	Automated partitions may represent populations rather than species Incipient allopatric speciation remains possible Add denser geographic sampling and non-geographic corroboration
Integrative recognition and synonymy within one complex		
Proposed adjudication rule		
Phylogenomics is evaluated with morphology, population structure, or biogeography	Taxa with conflicting historical names and genomic partitions	Different lineages can legitimately receive different taxonomic outcomes Evidence classes may be incomplete or non-independent Make lineage-specific decisions rather than applying one threshold to the complex
Species count rises with locus number		
Evidence-supported warning		
Additional loci increase automated partitions	Phylogenomic delimitation	More data can increase numerical resolution without resolving rank True shallow species may also require many loci Test partition stability and compare with independent taxonomy
Discordance remains non-identifiable		
Proposed adjudication rule		
Multiple demographic histories fit the available observations	Sparse sampling or weakly discriminating data	A mechanism-specific taxonomic conclusion is premature Better data may still fail to separate recent processes Retain explicit uncertainty instead of forcing splitting or lumping

False splitting and false lumping

False splitting and false lumping are reciprocal consequences of the same inferential mistake: treating one evidence class or one model-conditioned partition as equivalent to species status. In the Bemisia tabaci cryptic complex, mitochondrial, nuclear, genomic, geographic, host-associated, and reproductive information do not carry identical delimitation signals; nuclear and genome-scale evidence can change interpretations suggested by mtDNA alone [32]. The implication is not that nuclear evidence is automatically decisive. Rather, discordance should trigger tests of evidence independence, demographic history, and sampling completeness before a lineage is named or collapsed.

Integrative studies show how this two-sided error can be reduced without pretending that all evidence classes have equal weight. In French Polynesian Pseudoloxops, molecular data were interpreted together with morphology, genital characters, host associations, and geography [33]. In Dichaetophora and related drosophilids, molecular phylogeny revised understanding of diversity and relationships, but phylogenetic placement remained analytically distinct from the decision to recognize species [34]. Classification changes in Pseudocoladenia butterflies

similarly demonstrate that lineage history and nomenclatural rank need not be one-to-one [35]. Evidence convergence is therefore most informative when the contributing datasets test different biological propositions rather than merely replicate correlated genetic signals.

False splitting is most plausible when geographically structured populations, marker-specific clades, or method-sensitive partitions are converted directly into named species. Allopatric Lepidoptera illustrate how geographic structure can remain difficult to classify as population versus species divergence [11]. MSC-based analyses can also strengthen population partitions without independently resolving species rank [19]. In Stylops, inferred species number was sensitive to locus number [31]. False lumping is most plausible when taxonomic conservatism dismisses reproducible differentiation because one marker is shared, gene flow occurs, or morphology remains similar. Neither error can be minimized by always preferring the more conservative species count.

The proposed validation architecture therefore treats splitting and lumping as alternative failure states reached after insufficient challenge of a candidate boundary. The two errors are not symmetrical in their observable evidence. False splitting can look statistically convincing because additional data can reinforce structured partitions, whereas false lumping can arise from insisting on concordance across evidence classes that evolve on different timescales. The appropriate comparison is not "more versus fewer species," but whether the proposed boundary explains the joint pattern better than a within-species structure hypothesis.

The architecture in **Figure 1** is needed because the same observed partition can move toward recognition, synonymy, or retained uncertainty depending on which analytical challenge it survives.

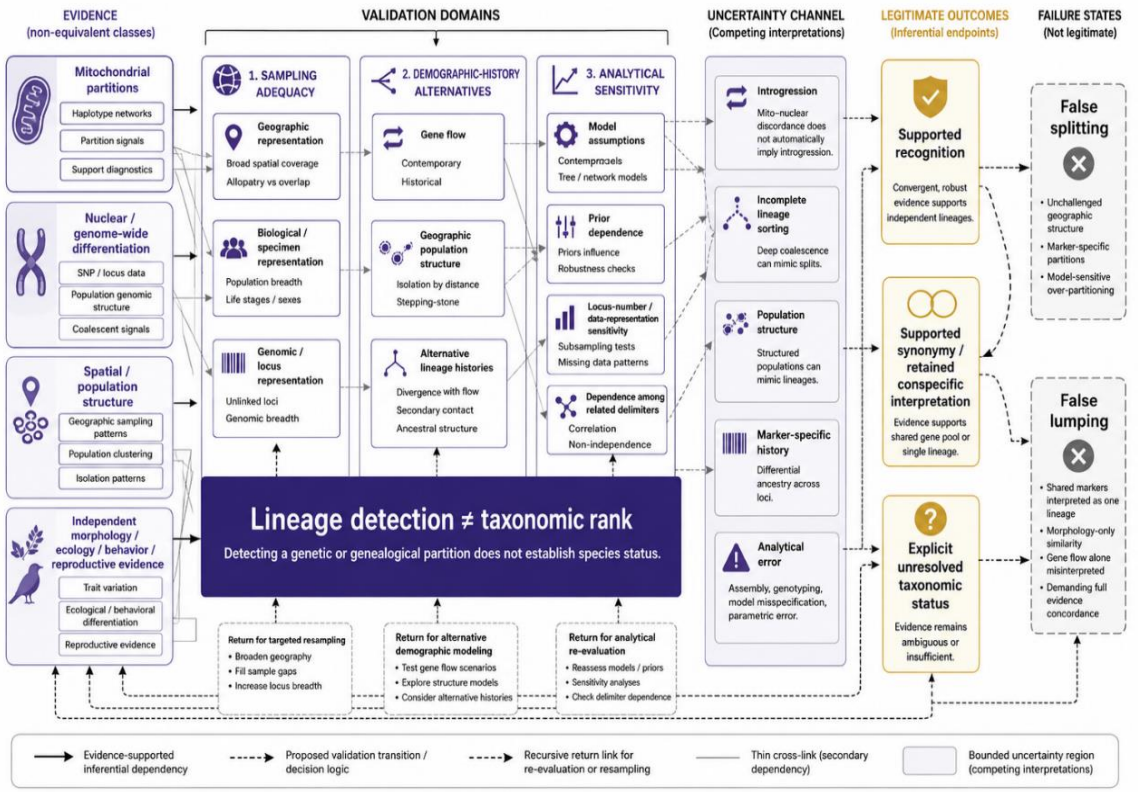


Figure 1. How sampling, demographic history, and evidence discordance redirect insect lineage partitions toward splitting, lumping, or retained uncertainty

Consequences for taxonomic decisions

Species delimitation becomes consequential when an analytical partition is converted into nomenclature, diagnostic practice, biodiversity inventories, or regulatory identification. Independent ecological information can strengthen a genomic species hypothesis when it measures a distinct axis of differentiation. In Sycophila parasitoid wasps associated with oak galls, phylogenomic lineages were evaluated alongside host preferences, providing ecological corroboration for otherwise cryptic diversity [36]. Host association, however, is neither complete nor exclusive in every lineage and therefore cannot by itself establish reproductive isolation.

Errors can propagate beyond nomenclature. In invasive-pest contexts, inadequately validated molecular-identification protocols can transmit taxonomic uncertainty into biosecurity decisions [37]. The literature does not justify a numerical estimate of that operational risk, but it establishes why diagnostic names should retain traceability to the evidence and assumptions supporting them.

Taxonomic action can also move in opposite directions after genome-scale analysis. Whole-genome evidence in the Papilio machaon complex distinguished species-level divergence from within-species geographic differentiation and informed taxonomic simplification [27]. Leptocircini phylogenomics clarified relationships while historical gene flow remained relevant to discordant systematic signals [10]. In Acromyrmex, integrative evidence supported different outcomes among lineages, including recognition and synonymy [30]. The appropriate action is therefore lineage-specific rather than threshold-specific.

Taxonomic revision also changes the units used in downstream comparative work. Molecular restructuring of Dichaetophora can alter which specimens and names are compared even though phylogeny does not dictate species rank [34]. Pseudocoladenia likewise illustrates that biogeographic history and nomenclatural rank remain separate analytical decisions [35]. **Table 3** separates taxonomic actions from the evidential boundaries that should constrain them.

Table 3. Taxonomic actions justified by different combinations of lineage evidence, validation, and unresolved alternatives

Boundary that remains and decisive check	Evidential trigger and warranted reading	Taxonomic decision state with evidential standing
Gene flow or shallow divergence may remain; no universal genomic threshold follows	Reproducible differentiation persists across suitable genomic analyses and is supported by independent evidence	Recognition of a distinct lineage
Verify sampling coverage and challenge plausible demographic alternatives	Species recognition may be justified for the focal lineage	Proposed decision rule
Real population structure must not be dismissed as biological noise	Apparent partitions track geography, morphotypes, or analytical settings more closely than independent species evidence	Synonymy or retained conspecific status
Demonstrate that alternative sampling or evidence classes do not recover independent lineage support	Named units may be combined when broader evidence supports within-species differentiation	Proposed decision rule
Introgression, incomplete lineage sorting, structure, and error remain non-identifiable	Mito-nuclear or cross-method discordance remains compatible with multiple histories	Unresolved taxonomic status
Acquire evidence capable of discriminating the live alternatives	Uncertainty should be retained explicitly	Proposed decision rule
Association does not itself establish reproductive isolation	Genomic differentiation aligns with independently measured host or ecological association	Ecologically corroborated cryptic lineage
Test repeatability, geographic scope, and independence of the ecological signal	Ecology can strengthen a species hypothesis	Evidence-supported boundary
Operational impact varies with marker, database, taxon, and regulatory setting	A taxonomic name is used for pest diagnosis, quarantine, or biosecurity	Operationally consequential identification
Maintain traceable diagnostic evidence and validate identification protocols	Taxonomic evidence quality becomes relevant to downstream decisions	Evidence-supported consequence

Critical evaluation of current practice

The central weakness in contemporary delimitation is not lack of analytical power but asymmetry between discovery and validation. Genomic delimiters can recover subtle population structure, whereas independent tests of whether that structure warrants species rank may remain sparse [1]. Recent genomic synthesis similarly emphasizes the analytical separation between discovering lineages and validating species hypotheses [2]. Numerical confidence can therefore increase faster than taxonomic confidence.

A second weakness is treating different sensitivity analyses as interchangeable. Sampling design measurably affects delimitation performance in a species-rich insect family [12]. Locus number can change automated species counts in phylogenomic data [31]. Prior sensitivity can also be made explicit rather than hidden behind one posterior result [25]. Stability across two methods may therefore still depend on the same geography, assignments,

or coalescent assumptions, while instability need not mean that a biological boundary is false. What matters is the diversity of plausible challenges a species hypothesis survives.

This criticism is not an argument against genomic delimitation. Contemporary insect systematics increasingly integrates genomic evidence with other information while recognizing discordance, missing data, and model limits [38]. The decisive question is whether independent evidence is used to test alternatives or merely accumulated after a preferred partition has been accepted.

Recommendations

First, candidate insect species should undergo a proposed analytical stress test before formal taxonomic action. A boundary should be challenged with demographic models that admit gene flow when biologically defensible [6], explicit evaluation of model violation [17], and prior sensitivity analysis [25]. Sampling perturbations should be chosen to expose the strongest plausible within-species alternative. Stability across these challenges is evidence of inferential robustness, not proof of species status.

Second, validation should be organized by evidential independence. Genomic validation should remain analytically distinct from lineage discovery [1]. Contact-zone evidence can test whether differentiation persists despite exchange [8]. Discordant mitochondrial and nuclear signals should be reconciled rather than assigning automatic precedence to either evidence class [32]. Independently measured ecological association can provide another corroborative axis [36]. Where such evidence is unavailable, that absence should remain visible rather than be replaced by agreement among related algorithms.

Third, taxonomic action should remain proportional to unresolved alternatives. Geographic structure that is over-partitioned by an automated method can justify synonymy after broader evidence is considered [29]. Integrative phylogenomics can support recognition of another lineage within a complex [30]. For operationally important taxa, transparent uncertainty is preferable to diagnostic certainty unsupported by the evidence [37]. The proposed approach therefore favors reversible, explicitly reasoned decisions over universal divergence thresholds or fixed species-count rules.

Operationally, this implies a sequence rather than a score. Researchers should state the biological proposition represented by a candidate species, identify the strongest competing within-species explanation, select sampling and analyses capable of distinguishing those propositions, and only then ask whether morphology, ecology, reproductive information, or geography supplies independent corroboration. If decisive alternatives remain observationally equivalent, the recommended result is an explicitly unresolved taxonomic hypothesis rather than a forced binary decision. This sequence is proposed as a reasoning and reporting discipline, not as a validated universal protocol.

Conclusion

Contemporary insect species delimitation is strongest when genomic resolution is matched by inferential restraint. Gene flow, geographic structure, sampling design, model assumptions, priors, and discordant evidence do not merely add noise; they determine which taxonomic interpretations remain defensible. The principal contribution of this review is a proposed validation architecture that separates lineage detection from species rank, treats false splitting and false lumping as reciprocal risks, and requires candidate boundaries to survive biologically relevant analytical challenges. It does not provide a universal species criterion. Its purpose is to make uncertainty, competing explanations, and the evidential basis of taxonomic action explicit. Accordingly, confidence should arise not from the numerical strength of a single partition, but from concordant survival of explicit challenges to sampling, demographic history, analytical assumptions, and evidential independence. Where those challenges cannot discriminate among live alternatives, preserving uncertainty is a scientifically informative result rather than a failure of delimitation.

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