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Resolution, Failure Modes, and Integrative Applications of DNA Barcoding in Contemporary Insect Systematics

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ABSTRACT

DNA barcoding has become a central component of insect identification, biodiversity inventory, reference-library construction and accelerated taxonomic workflows, yet its evidential meaning changes substantially according to the question being asked. A COI sequence may support specimen identification, reveal discordance requiring taxonomic investigation, generate a preliminary molecular cluster, or contribute to species delimitation, but these functions are not logically interchangeable. This evidence-mapping review examines contemporary insect-systematics literature through the linked problems of barcode resolution, reference-library dependence, biological discordance and integrative use. Across taxonomically and geographically diverse applications, high identification performance frequently coexists with barcode sharing, deep intraspecific structure, algorithm-dependent molecular operational taxonomic units and incompletely sampled reference libraries. The evidence therefore does not support a universal judgment that barcoding either resolves or fails to resolve insect species. Instead, resolution is conditional on taxon history, sampling design, reference quality, marker properties and the inference being attempted. A proposed distinction is developed between identification concordance, cluster concordance and delimitation sufficiency. Biological processes such as introgression and incomplete lineage sorting are further separated from nuclear mitochondrial sequences, sequencing artefacts and reference-data errors because superficially similar barcode discordances require different tests. The resulting evidence map positions DNA barcoding most strongly as a scalable identification and hypothesis-generation system whose taxonomic force increases when molecular patterns are adjudicated against independently informative morphology, geography, ecology, life-history information, additional loci and curated vouchers.

Keywords: COI barcoding, Insect systematics, Species delimitation, Reference libraries, Integrative taxonomy, Mitochondrial discordance

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Introduction

DNA barcoding occupies an unusually ambiguous position in contemporary insect systematics. The same cytochrome *c* oxidase subunit I (COI) sequence can be used to assign an unknown specimen to a reference, group specimens into molecular operational taxonomic units, expose conflict with an existing classification, or contribute to a formal species hypothesis. These operations differ in evidential strength. Zamani *et al.* argued

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specifically that barcode clusters may be useful grouping statements without being sufficient, by themselves, to justify species descriptions [1]. The central problem is therefore not whether barcodes are useful, but what conclusion a given barcode pattern is entitled to support.

That distinction becomes increasingly important as insect collections scale beyond the capacity of conventional specimen-by-specimen taxonomic workflows. Large-Scale Integrative Taxonomy uses inexpensive barcode data to construct preliminary species hypotheses and strategically directs more costly validation data toward cases requiring adjudication [2]. This approach treats molecular clustering as a triage mechanism rather than an automatic nomenclatural endpoint. Reanalysis of barcode-centred minimalist taxonomy has similarly questioned the equation of Barcode Index Numbers (BINs) with named species and highlighted the consequences of limited data accessibility and insufficient independent diagnosis [3].

Empirical discordance reinforces this inferential caution. In Sri Lankan Sericini, different COI-based delimitation procedures did not consistently recover the same molecular units or the same correspondence with morphospecies; both splitting and lumping occurred [4]. Thus, disagreement can arise even before biology is invoked: the same sequence dataset may yield different partitions because analytical procedures define boundaries differently.

This review maps that problem across four linked dimensions. First, barcode resolution concerns the correspondence among sequences, molecular clusters and independently identified taxa. Second, reference resolution concerns whether the relevant taxon, geography and correctly identified voucher actually exist in the comparison library. Third, discordance mechanism concerns whether an unexpected pattern reflects introgression, lineage sorting, nuclear mitochondrial copies, population structure, analytical artefact or taxonomic error. Fourth, integrative resolution concerns how barcode evidence changes meaning when combined with independent characters. The review therefore proposes that barcode performance should be judged against the inference being attempted, rather than through a single universal measure of "success."

Materials and Methods

Search, evidence coding, and map construction

The evidence map was restricted to peer-reviewed literature published from 2017 through 2022 and focused on insect DNA barcoding in systematic, taxonomic, biodiversity-inventory and identification contexts. Search logic combined terms for *DNA barcoding*, *COI*, *cytochrome oxidase I*, *Barcode Index Number*, *molecular operational taxonomic unit*, *species delimitation*, *reference library*, *voucher*, *misidentification*, *introgression*, *incomplete lineage sorting*, *NUMT*, *mini-barcode*, *integrative taxonomy*, and major insect-order or family names. Methodological studies were retained when their claims altered interpretation of insect barcode evidence, particularly single-locus delimitation, reference construction, sequence length, high-throughput sequencing and specimen-linked multilocus approaches.

Evidence was coded at the level of the claim rather than treating each publication as a homogeneous vote for or against barcoding. For each relevant analysis, coding distinguished taxonomic scope, geography, specimen or life-stage coverage, barcode/reference construction, molecular clustering method, morphological or other comparator, concordant identification, barcode sharing, within-species structure, inferred mechanism and limitations acknowledged by the authors. Statements about species identification were not recoded as evidence of species delimitation unless the study tested that inference explicitly. Conversely, molecular clusters that disagreed with named species were recorded as discordant observations rather than automatically interpreted as cryptic species or taxonomic error.

The map further separated observed patterns from mechanistic interpretation. Shared haplotypes, non-monophyly, deep within-species divergence and multiple molecular clusters were treated as observations. Introgression, incomplete lineage sorting, nuclear mitochondrial copies, historical isolation or misidentification were coded as explanations only where supported or explicitly evaluated. The article's higher-order categories—identification concordance, cluster concordance and delimitation sufficiency—are proposed synthesis terms and should not be interpreted as externally validated classification states.

Results and Discussion

Taxonomic and geographic coverage

The mapped literature demonstrates broad application of COI barcoding but uneven taxonomic and geographic depth. Chironomidae from the Lake Skadar region provide a useful example of intensive regional library construction: adults and pre-imaginal stages were incorporated, and the regional dataset was evaluated against a much larger European barcode resource rather than interpreted only within the sampled basin [5]. This design illustrates that apparent resolution is partly a function of the comparison universe.

Lepidopteran surveys in Xinjiang similarly used barcoding to characterize a regional fauna while comparing multiple molecular delimitation procedures [6]. Their relevance to an evidence map extends beyond regional inventory because the study exposes the analytical dependence of inferred molecular diversity. In contrast, the Finnish arthropod initiative was designed at a national scale, constructing a molecular identification resource across a substantial fraction of the country's known insect fauna and explicitly testing how expansion of the reference library changed identification performance [7].

Geographic gaps remain conspicuous outside intensively barcoded European and North American faunas. A large survey across Pakistan recovered extensive barcode diversity across numerous insect orders but achieved progressively lower taxonomic assignment toward genus and species levels, demonstrating that sequencing coverage and Linnaean annotation coverage are different properties of a reference system [8]. The presence of a molecular cluster therefore cannot be interpreted as equivalent to the presence of a taxonomically resolved reference.

Technological development is also altering which insect assemblages can enter reference-building programmes. DiversityScanner links individual Malaise-trap specimens to images, measurements and barcode-ready positions, thereby preserving specimen-level association while automating a bottleneck that otherwise separates high-throughput collection from taxonomic processing [9]. Portable amplicon-sequencing protocols extend sequence-based identification into settings without conventional molecular infrastructure, although portability addresses laboratory accessibility rather than the correctness of the taxonomic reference against which sequences are interpreted [10].

Taxonomic saturation does not eliminate biological discordance. The North American butterfly barcode library covered most recognized species yet still detected barcode sharing, non-monophyly and marked within-species sequence variation in subsets of the fauna [11]. Consequently, well-sampled and poorly sampled faunas pose different problems: the latter can fail because the reference is missing, whereas the former can reveal genuine mitochondrial patterns that do not map one-to-one onto currently recognized species.

Together, these studies support an evidence landscape dominated by regional or national reference-building projects, order- or family-specific evaluations and technologically enabled scaling. They do not support extrapolating one order's resolution rate to insects generally. Geographic coverage, taxonomic expertise, developmental-stage representation and library completeness remain properties of individual evidence systems rather than constants of COI itself.

Barcode resolution

Barcode resolution is most informative when divided into three questions: can a sequence identify a specimen against a validated reference; does molecular clustering correspond to an independently formulated taxonomic unit; and is the resulting evidence sufficient to delimit or name a species? Treating these questions as interchangeable is the principal inferential failure addressed by this review.

The controversy surrounding accelerated barcode-centred taxonomy makes the distinction explicit. Zamani *et al.* cautioned that the omission of critical descriptive and taxonomic information can undermine attempts to accelerate species description [12]. By contrast, the Costa Rican braconid revision demonstrated the practical extreme of a minimalist strategy, using consensus COI sequences and BIN assignments as major components of diagnoses across hundreds of parasitoid-wasp taxa [13]. These papers therefore represent contrasting positions on what barcode information can legitimately carry at the nomenclatural endpoint; their disagreement should not be reframed as disagreement over whether COI sequences contain useful information.

Sequence production itself can be highly scalable without resolving this inferential issue. ONTbarcoder generated nanopore barcodes that closely matched conventional sequence data and was designed for rapid, inexpensive biodiversity processing [14]. Increased throughput improves access to molecular observations, but it does not independently validate the species boundary represented by a cluster.

Empirical libraries show why a conditional interpretation is necessary. Central and Northern European Odonata reference data included adults and larvae and revealed both useful identification structure and taxa with unusually

small interspecific distances or deep intraspecific variation requiring further investigation [15]. In Loire Valley bees, most sampled species formed diagnostically useful molecular groups, while a smaller set contained deep conspecific lineages or shared BINs [16]. These are not opposite outcomes; they are different states within the same identification system.

A second source of apparent resolution failure is model behaviour. Species-delimitation methods based on genetic structure can over-split geographically structured populations when assumptions concerning population structure and sampling are violated [17]. Although that result is methodological rather than a COI-specific insect demonstration, it places an important boundary around interpreting molecular subdivision as species evidence.

Barcode length adds another conditional dimension. Across large empirical and database datasets, moderately sized mini-barcodes could retain substantial species-sorting information relative to full-length barcodes, whereas very short fragments performed less consistently [18]. Resolution therefore depends not simply on the label "COI barcode" but on which fragment, which reference library, which taxonomic group and which inference criterion are being evaluated.

This review consequently proposes five resolution states for evidence mapping rather than a binary success/failure classification: *reference-concordant identification*, *barcode sharing*, *deep within-species partitioning*, *method-contingent partitioning* and *insufficient-reference resolution*. These states describe what the data permit investigators to say next; none is itself a species-delimitation verdict.

Because those states differ simultaneously in observation, taxonomic context and the test needed to adjudicate them, a prediction-and-test ledger is more informative than a single percentage of barcode "accuracy."

Table 1. Barcode resolution states and the evidence tests required before molecular partitions acquire taxonomic meaning

Identification and biological discordance			
Resolution criterion	**Reference-concordant identification†**	**Barcode sharing†**	**Deep within-species partitioning†**
Barcode construct	Query-to-reference correspondence	Shared or non-exclusive COI sequence/cluster	Multiple divergent haplotypes or clusters within one taxon
Observed pattern	Barcode assignment agrees with independently identified reference material	Two or more recognized taxa lack unique barcode separation	Conspecific samples form strongly differentiated barcode units
Taxonomic force allowed	Supports identification within the tested reference domain; does not independently delimit species	Requires independent evidence before synonymy, lumping or identification failure is inferred	Generates a taxonomic hypothesis; does not establish cryptic species
Reference domain	Curated regional or taxon-specific libraries	Closely related named taxa	Geographically or genetically structured named taxa
Quality limitation	Geographic or taxonomic library may be incomplete outside the validated scope	Introgression, lineage sorting, recent divergence, taxonomy or sampling can produce similar patterns	Population structure, overlooked diversity, sampling gaps and erroneous identification are alternatives

Method, fragment, and library constraints			
Resolution criterion	**Method-contingent partitioning†**	**Fragment-conditioned resolution†**	**Insufficient-reference resolution†**
Barcode construct	mOTU/BIN or model-based cluster	COI fragment information content	Query with absent/inadequate named match
Observed pattern	Different methods split or lump the same specimens differently	Moderate-length fragments can retain substantial sorting information; very short fragments may lose resolution	Sequence is recoverable but species-level reference assignment is unavailable
Taxonomic force allowed	Discordance should trigger adjudication rather than selection of the preferred cluster by convenience	Sequence length is a validation variable, not a universal quality ranking	Record as reference limitation rather than biological barcode failure
Reference domain	Datasets analysed by multiple delimitation procedures	Full-length versus mini-barcode workflows	Poorly represented taxa or regions

Quality limitation	Algorithmic assumptions and thresholds influence inferred boundaries	Performance depends on position, taxon and comparator	Database completeness and annotation, rather than marker biology, constrain interpretation

†Proposed evidence-mapping classification developed in this review. It organizes patterns reported in the reviewed literature but is not an independently validated species-delimitation system.

Reference-library limitations

A barcode identification system is relational: a query sequence can only perform as well as the specimens, names and geographic variation represented in its reference environment. High congruence in a well-curated national resource therefore measures both properties of the marker and properties of library construction. In British bees, a high-throughput workflow obtained broad reference coverage and strong correspondence with morphological concepts, while molecular delimitation nevertheless produced both split and lumped entities [19]. Reference completeness can improve identification without eliminating biological or analytical discordance.

The same distinction is critical in community and metabarcoding applications. Methodological guidance for entomological metabarcoding emphasizes that extraction, amplification, contamination and bioinformatic decisions can affect species detection [20]. Such downstream biases should not be confused with taxonomic reference failure, although the two may interact: an apparently absent species can reflect failure to recover its sequence, absence of a suitable reference, or a genuinely absent organism.

Targeted Central European carabid libraries illustrate the value of preserving that distinction. Most examined *Pterostichus* and allied taxa showed correspondence between BINs and recognized species, but the same dataset also contained barcode sharing and elevated within-species divergence [21]. A reference library can therefore be simultaneously useful for routine identification and diagnostically valuable because its exceptional cases expose unresolved taxonomic or evolutionary questions.

Coccinellidae research further shows that a library is not simply a collection of full-length modern sequences. Mini-barcoding primers recovered barcode information from older museum material and were combined with public sequences and multiple delimitation approaches [22]. Museum integration expands temporal and taxonomic coverage, yet threshold performance demonstrated for one beetle family cannot be assumed for other insect clades.

Moving beyond a single locus is one route to reducing dependence on one mitochondrial history. Multiplexed workflows have generated specimen-linked multilocus barcode data across diverse arthropod assemblages, retaining individual-level information while increasing molecular dimensionality [23]. Such approaches do not make taxonomic interpretation automatic, but they provide independent sequence evidence capable of testing whether a COI partition recurs elsewhere in the genome.

Reference-library quality should therefore be evaluated along at least four independent dimensions: coverage (is the candidate taxon represented?), annotation validity (is the reference name defensible?), geographic representation (does sampling span relevant mitochondrial variation?) and specimen traceability (can the molecular record be reconnected to a voucher?). A fifth dimension, analytical transparency, concerns whether sequence and clustering decisions can be independently inspected. These dimensions are proposed as an interpretive checklist rather than a numerical quality score.

Introgression, NUMTs, and incomplete lineage sorting

Not all departures from one-species–one-barcode correspondence have the same origin. A central interpretive error is therefore to diagnose mechanism from pattern alone. Large Diptera reference libraries have revealed extensive molecular diversity while simultaneously exposing cases requiring additional taxonomic investigation [24]. Such libraries are valuable precisely because discordant records can be revisited; the exceptional barcode is a signal to test, not a self-interpreting explanation.

High-throughput sequencing adds a second layer of uncertainty because molecular anomalies can arise during sequence production as well as through organismal history. Rapid nanopore barcoding has demonstrated the feasibility of processing specimen-rich samples at large scale [25]. Scale, however, increases the importance of recognising sequence-quality problems before attributing unexpected divergence to biological structure.

Single-locus delimitation scholarship provides the corresponding inferential guardrail: mitochondrial partitions can be informative species hypotheses, but their interpretation depends on sampling, the delimitation method and the biological processes capable of decoupling a gene history from a species history [26]. A COI tree is therefore neither meaningless nor automatically a species tree.

Technical replication and alternative sequencing workflows help separate some of these categories. Earlier MinION pipelines showed that high-throughput specimen barcodes can be generated and checked against established sequencing approaches [27]. Concordance across technical methods reduces the plausibility of a platform-specific artefact but does not distinguish among introgression, ancestral polymorphism or other biological causes of mitochondrial sharing.

Geography introduces another critical boundary. Comparisons of European and South Siberian Lepidoptera found that large geographic separation can coexist with relatively small barcode divergence in many taxa [28]. Conversely, deep structure within a named species may reflect historical geographic subdivision without necessarily constituting evidence for multiple species. Geographic distance therefore cannot be translated into either expected barcode distance or taxonomic rank by a universal rule.

Introgression is most defensible as an explanation when mitochondrial sharing is accompanied by independent evidence that otherwise differentiated lineages have exchanged genes. Incomplete lineage sorting remains plausible when ancestral mitochondrial variants persist across recently diverged lineages, especially where nuclear and mitochondrial histories disagree. NUMTs represent a fundamentally different error class: nuclear copies of mitochondrial sequence can be amplified or sequenced and mistaken for orthologous mitochondrial COI. Sequence anomalies, stop codons, frame disruption, unexpected divergence, replicate inconsistency or disagreement among amplicons can raise suspicion, but none is individually a universal diagnostic criterion.

The proposed synthesis is therefore a discordance-adjudication rule: first establish whether the anomaly is reproducible; next determine whether it is reference-dependent; then ask whether independent loci, geography, morphology or population sampling discriminate among biological alternatives. This ordering prevents a taxonomic conclusion from being chosen before the mechanism generating the discordance has been tested.

The distinctions are sufficiently consequential that they are better expressed as a decision matrix than as a single category of "barcoding error."

Table 2. Taxonomic interpretation of superficially similar COI discordances requires different evidential tests

Biological histories		
Putative introgression		*Evidence-dependent mechanism*
Signature in the focal taxon	Closely related taxa with mitochondrial sharing despite other differentiation	Shared/non-exclusive COI combined with evidence of lineage contact or discordance from independent characters
Barcode-stage interpretation	Mitochondrial history is inconsistent with simple reciprocal isolation	
Competing biological or technical cause	ILS, misidentification and inadequate sampling can mimic sharing	
Independently sampled nuclear loci, geographic contact tests and expanded population sampling		**ADJUDICATION TEST**
Putative incomplete lineage sorting		*Evidence-dependent mechanism*
Signature in the focal taxon	Recent or structured divergence	Mitochondrial lineages fail to sort according to candidate species boundaries
Barcode-stage interpretation	COI alone does not recover the inferred species history	
Competing biological or technical cause	Introgression and population structure can produce similar gene-tree discordance	
Multilocus species-history analysis with adequate population sampling		**ADJUDICATION TEST**
Sequence artefacts		
Putative NUMT		*Technical/biological discrimination required*
Signature in the focal taxon	Specimen yields anomalous COI-like sequence	Unexpected sequence structure, replicate inconsistency or mitochondrial/nuclear conflict
Barcode-stage interpretation	Sequence identity as orthologous mitochondrial COI is uncertain	
Competing biological or technical cause	Genuine divergent haplotype or sequencing error remains possible	

Re-amplification, alternative primers, translation/frame inspection and independent mitochondrial evidence		**ADJUDICATION TEST**
Platform or amplicon artefact		*Technical validation state*
Signature in the focal taxon	High-throughput or mini-barcode workflow	Discordant sequence not reproducible across technical routes
Barcode-stage interpretation	Barcode should not be interpreted taxonomically until technically validated	
Competing biological or technical cause	Contamination, base-calling, amplification error or specimen mix-up	
Replicate extraction/sequencing and comparison with validated reference sequence		**ADJUDICATION TEST**
Population structure and unresolved sharing		
Geographic mitochondrial structure		*Context-dependent signal*
Signature in the focal taxon	Broadly distributed named species	Deep or spatially patterned within-species COI variation
Barcode-stage interpretation	Population differentiation or a taxonomic hypothesis may be reported	
Competing biological or technical cause	Cryptic species, isolation by distance and undersampling are alternatives	
Denser geographic sampling plus morphology, ecology and independent loci		**ADJUDICATION TEST**
Barcode sharing without mechanistic evidence		*Proposed conservative state†*
Signature in the focal taxon	Two named taxa occupy one COI cluster/haplotype set	Non-exclusive mitochondrial barcode pattern
Barcode-stage interpretation	Identification ambiguity is established for the tested barcode/reference combination	
Competing biological or technical cause	Mechanism is unresolved	
Treat mechanism as unknown until independent evidence discriminates alternatives		**ADJUDICATION TEST**

†The "barcode sharing without mechanistic evidence" state is proposed here to prevent an observed mitochondrial pattern from being prematurely labelled introgression, incomplete lineage sorting or taxonomic synonymy.

Misidentification and voucher problems

Barcode accuracy depends on the biological specimen behind the sequence. An incorrectly named reference can propagate a confident but incorrect identification, whereas an unvouchered sequence prevents later reassessment when taxonomy changes. Specimen linkage is particularly important when DNA barcoding connects morphologically dissimilar life stages. Rapid adult–larva matching demonstrates how sequence identity can unite developmental forms that would otherwise be difficult to associate, but the strength of that association depends on preserving traceable specimen and sequence records [29].

Entomological collections consequently remain infrastructure for molecular systematics rather than historical repositories displaced by sequencing. Digitisation, imaging, genomic sampling and specimen-linked metadata can make collections increasingly reusable, but their value depends on persistent connections among names, specimens, determinations and derived data [30]. Integrative taxonomy of difficult complexes likewise shows that morphology, molecular evidence, geography and other characters may each expose errors invisible to another evidence class [31].

Sequence-quality control cannot substitute for taxonomic quality control. Semi-automated barcode processing can detect contamination, sequence anomalies and inconsistencies between molecular assignments and expected taxonomy, thereby identifying records requiring re-examination [32]. Such procedures flag discordance; they do not establish whether the sequence, voucher identification or current taxonomy is wrong.

This review therefore proposes a distinction between sequence-validity failure, voucher-identity failure, annotation failure and traceability failure. Their consequences differ sufficiently that collapsing them into "database error" obscures the corrective action required.

The resulting context–mechanism–outcome register clarifies which failures can be repaired computationally and which require renewed taxonomic examination.

Table 3. How specimen and annotation failures propagate through barcode identification

Failure detection			Propagation	Repair	Inferential guardrail
Failure locus	**Observable fault**	**Barcode-system location**	**Downstream consequence**	**Corrective action**	**What the fault does not establish**
Sequence and voucher integrity					
Sequence-validity failure†	Poor-quality, contaminated or inconsistent sequence	Reference or query sequence	Molecular assignment becomes unreliable	Re-sequence or independently validate	Does not establish specimen misidentification
Voucher-identity failure†	Molecular placement conflicts with independently reviewed morphology or taxonomy	Named reference specimen	Incorrect name may propagate to queries	Re-examine voucher and determination	Molecular conflict alone does not identify the correct name
Annotation and traceability					
Annotation failure†	Sequence–specimen link is retained but metadata/name are outdated or incorrect	Database record	Search result may be technically precise but taxonomically misleading	Curate metadata while retaining specimen history	Taxonomic revisions may legitimately alter names
Traceability failure†	Reference cannot be physically reassessed	Sequence lacking recoverable specimen linkage	Subsequent conflict cannot be adjudicated directly	Prefer newly generated, vouchered replacement records	Not every legacy sequence is erroneous
Life-stage linkage					
Life-stage linkage uncertainty†	Matching barcode links unlike developmental forms	Adult–immature association	Supports association within the tested molecular context	Add geography, morphology or additional loci where needed	Shared barcodes among close taxa can weaken exclusivity

†Proposed failure-state terminology used in this review.

Integrative uses beyond stand-alone delimitation

The strongest role for DNA barcoding emerges when it reduces uncertainty without being asked to carry the entire taxonomic inference. Barcode-linked reference libraries can connect adults and immature stages, accelerate sorting of bulk material, expose anomalous specimens and direct scarce specialist effort toward discordant cases. German Ephemeroptera, Plecoptera and Trichoptera reference development illustrates how extensive taxonomically curated barcode libraries can support identification while simultaneously identifying taxa for which mitochondrial evidence remains unresolved [33].

Integrative application therefore changes the question from “Does COI define the species?” to “What uncertainty does COI remove, and what uncertainty remains?” Morphology can test whether barcode partitions correspond to diagnostic phenotype; geography can reveal spatial structure; ecological or host data can identify biologically structured lineages; nuclear loci can test whether mitochondrial partitions recur independently; and vouchers permit reinterpretation when classifications change.

The article proposes a conditional barcode-adjudication architecture in which barcode evidence can occupy three roles: identification support, discordance detection, or candidate-lineage generation. Movement from any of these roles to a taxonomic conclusion requires an explicit interpretation boundary rather than an automatic transition.

Because this logic is nonlinear—discordant evidence can return a case to reference checking, specimen reassessment or additional character sampling—it is compressed below as a mechanism-and-boundary map rather than a linear pipeline and is shown in **Figure 1**.

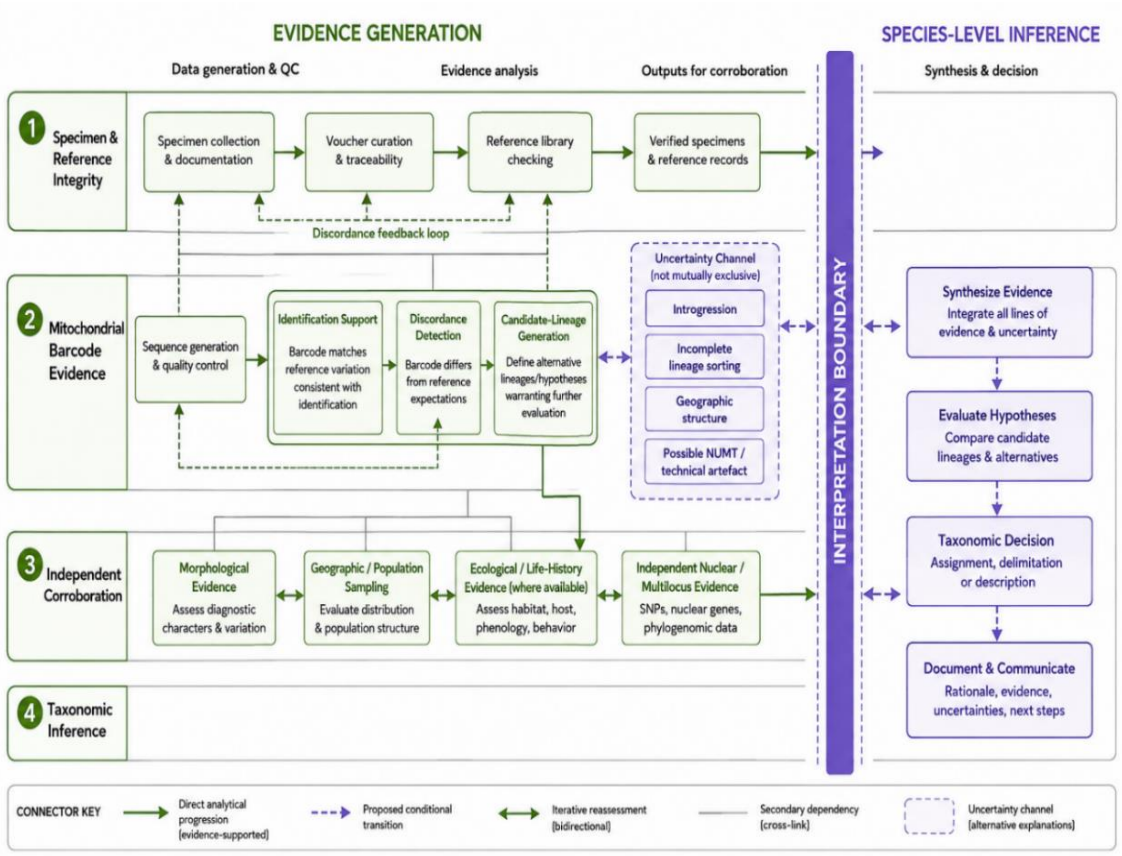


Figure 1. Barcode evidence changes taxonomic force only after crossing explicit corroboration boundaries

Evidence map and research gaps

The mapped literature is strongest where curated regional faunas, taxonomic expertise and specimen-linked sequencing coincide. It is weaker where reference libraries are geographically sparse, species-rich lineages lack stable names, immature stages are poorly represented, or barcode discordance has not been tested against independent loci and morphology.

Four gaps recur across the synthesis: inadequate geographic sampling within widespread taxa; insufficient distinction between reference failure and biological barcode sharing; limited validation of discordant mitochondrial clusters with independent evidence; and weak reporting of voucher traceability in some accelerated workflows. These gaps prevent a universal estimate of "barcode success."

The coverage-and-gap map therefore prioritises missing tests, not merely missing sequences.

Table 4. Evidence gaps that most directly constrain interpretation of insect barcode resolution

	Sampling coverage	
Evidence-map gap	**Geographic under-sampling†**	**Reference incompleteness†**
Presently observable	Sparse regional haplotypes	Query lacks validated species match
Interpretation constrained	Depth of within-species variation	Identification performance
Typical context	Widespread species	Poorly catalogued faunas
Principal uncertainty	Local structure versus lineage separation	Missing reference versus novel lineage
Priority test	Population-scale geographic sampling	Curated voucher-backed library expansion
	Validation independence	

Evidence-map gap	Single-locus discordance†	Weak voucher traceability†	Method-sensitive clustering†
Presently observable	Mitochondrial conflict	Sequence cannot be readily rechecked	Alternative algorithms yield different units
Interpretation constrained	Species-boundary inference	Correctness of reference identity	Number and limits of candidate lineages
Typical context	Shared or split COI clusters	High-throughput or legacy datasets	Automated delimitation
Principal uncertainty	Introgression, ILS or structure	Annotation versus specimen error	Model/threshold dependence
Priority test	Independent loci and integrative characters	Recoverable vouchers and metadata provenance	Cross-method comparison plus independent validation

†Proposed evidence-gap categories derived from the synthesis.

Contemporary insect barcoding is best understood as an inference system with several possible failure points rather than as a single diagnostic test. The evidence reviewed here shows that excellent specimen identification can coexist with mitochondrial sharing, deep intraspecific structure and incomplete taxonomic annotation. These conditions are compatible because they occur at different inferential levels.

A central implication is that barcode resolution is question-dependent. The evidence needed to assign a specimen to a curated reference is weaker than that required to infer a species boundary. Likewise, discovery of a molecular partition is not equivalent to demonstrating reproductive, evolutionary or taxonomic independence. This distinction parallels broader concerns in species-delimitation theory: genetic-structure methods can delimit population structure rather than species when their assumptions are interpreted taxonomically without sufficient biological corroboration [34]. Species delimitation under gene flow further demonstrates that historical exchange and lineage divergence cannot always be represented by a simple bifurcating boundary inferred from one locus [35].

The proposed synthesis therefore separates three evaluative layers. Identification concordance asks whether the barcode places a specimen with a validated reference. Cluster concordance asks whether independently analysed specimens form molecular units corresponding to an external taxonomic hypothesis. Delimitation sufficiency asks whether the accumulated evidence justifies a species-level conclusion. Progression through these layers is conditional rather than automatic.

This framing also changes the meaning of failure. Barcode sharing is a failure of exclusive COI identification only within the tested reference context; it is not necessarily a failure of taxonomy. Deep intraspecific divergence can signal overlooked diversity, population structure, historical isolation or error. Missing library coverage is a reference-system limitation rather than evidence that COI lacks biological variation. NUMTs and technical artefacts require sequence validation, whereas introgression and incomplete lineage sorting require biological evidence beyond the anomalous mitochondrial pattern.

The practical implication is not that barcoding should retreat from insect systematics. Its scalability is precisely what makes discordance visible across specimen numbers inaccessible to many traditional workflows. The stronger strategy is to use that scalability to allocate taxonomic effort efficiently: routine concordant assignments can remain routine, while unstable clusters, shared barcodes, unusual within-species structure and questionable reference records become explicit targets for integrative reassessment.

Research priorities consequently lie less in searching for a universal COI threshold than in strengthening inferential transitions. Priority should be given to geographically broader voucher-linked sampling, reproducible reference curation, explicit reporting of analytical sensitivity, multilocus testing of mitochondrial discordance, and designs capable of distinguishing population structure from candidate species boundaries. These steps would improve what barcode evidence can legitimately support without requiring it to become a universal surrogate for integrative taxonomy.

Conclusion

DNA barcoding has transformed the scale at which insect specimens can be identified, organised and compared, but its taxonomic meaning remains conditional. The decisive distinction is between detecting molecular structure

and interpreting that structure as a species boundary. Reference quality, geographic sampling, analytical method, mitochondrial history, sequence validity and voucher traceability can each alter the apparent result. DNA barcodes are therefore most defensible as scalable evidence for identification, discordance detection and candidate-lineage generation. Their strongest systematic application occurs when these functions are connected to explicit corroboration and interpretation boundaries rather than treated as automatic species delimitation.

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