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Environment drives phenotypic plasticity: a geometric-morphometric re-evaluation of the *Cryptotaenia japonica* complex (Apiaceae)

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Abstract

The study provides quantitative evidence refuting the traditional tripartite infraspecific classification of *Cryptotaenia japonica* complex (Apiaceae) in East Asia, whose taxonomic status is controversial because its leaf-shape variation has historically been divided into three infraspecific forms (f. *japonica*, f. *dissecta*, f. *pinnatisecta*) based on qualitative assessments, and it is unclear if these forms represent discrete lineages or a continuous response to environmental gradients. The authors analyzed 1,061 leaf specimens from 72 East Asian populations and 9 North American *C. canadensis* samples using geometric morphometrics and fractal dimension analysis. Unsupervised multivariate approaches (PCA, hierarchical clustering, silhouette analysis) were applied to assess the intrinsic structure of the data without any a priori taxonomic assignment. The results revealed a continuous morphological gradient, with PCA capturing 68.63% of the total variance. The low Adjusted Rand Index (ARI = 0.088) statistically refuted the three-form classification. Generalized additive modelling (GAM) identified a significant nonlinear relationship between leaf complexity (PC1) and altitude (adj. R^2= 0.0559, $P < 0.001$), with an inflection point occurring at 850 m s.l.m. These findings demonstrate that the three traditional infraspecific forms are not discrete taxa, but rather arbitrary segments along a continuous altitudinal phenotypic cline. Multivariate analyses statistically reject the tripartite classification, leaf complexity increases non-linearly with elevation (threshold ~850 m s.l.m), and the phenotypic contrasts between East Asian *C. japonica* and North American *C. canadensis* suggest divergent evolutionary strategies. Consequently, we propose reducing f. *dissecta* and f. *pinnatisecta* to synonyms under *C. japonica*, reflecting altitudinal morphological variation within a single, polymorphic taxon.

Key words: *Cryptotaenia japonica* complex, Geometric morphometrics, Phenotypic plasticity, Continuous variation, Fractal dimension, Altitudinal cline, Plant taxonomy

Introduction

The classification of infraspecific taxa in widespread herbaceous species historically depends on qualitative assessments of leaf morphology (Díaz *et al.*, 2022). In species with broad geographic ranges, morphological variation often manifests as a continuous gradient or cline driven by phenotypic plasticity (Nicotra *et al.*, 2011). *Cryptotaenia* DC. (Apiaceae) comprises 5–6 species worldwide (Pan *et al.*, 2005), including *Cryptotaenia japonica* Hassk., an East Asian endemic that exhibits a notable intercontinental disjunction with North American relatives (Spalik and Downie, 2007). Due to its recognized intrinsic value (Okuno *et al.*, 2017; Wu *et al.*, 2014), *C. japonica* is characterized by significant morphological variation and exists as a series of geographical morphological variants (Shan *et al.*, 1985) displaying an almost continuous range of forms (Pan *et al.*, 2005). These overlapping morphological traits pose a persistent challenge for taxonomic delimitation, necessitating a more precise quantitative approach to resolve the complex variation patterns within the species.

The application of unsupervised clustering and geometric morphometrics offers a critical framework for resolving persistent taxonomic ambiguities (Bellin *et al.*, 2022). Within the *C. japonica* complex, three primary morphological

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forms are traditionally delineated based on leaf architecture: *C. japonica* f. *japonica*, *C. japonica* f. *dissecta* (Y.Yabe) Hara, and *C. japonica* f. *pinnatisecta* S. L. Liou (Liou, 1990). The diagnostic features of f. *dissecta* include median and lateral leaflets that are pinnatifid or deeply lobed with sharp marginal serrations (Shan *et al.*, 1985), whereas f. *pinnatisecta* is characterized by pinnately divided leaflets, with the median leaflet possessing a ~1 cm petiolule and lanceolate segments (Liou, 1990). Despite these formal descriptions, a fundamental tension exists between the localized discrete classification of these forms and the broad-scale continuous variation observed in nature. Field evidence suggests that these diagnostic traits often yield to transitional morphologies along environmental gradients or co-occur within single populations, indicating a phenotypic continuum that challenges the validity of traditional categorical boundaries.

Geometric morphometrics has fundamentally transformed quantitative shape analysis by preserving the geometric integrity of biological structures while effectively filtering out non-shape variations (Yucedag *et al.*, 2026). The core strength of this approach lies in the use of Procrustes superimposition, which isolates pure shape by removing the confounding effects of size, location, and orientation (Mitteroecker and Gunz, 2009). This provides a rigorous quantitative framework for characterizing complex phenotypes, utilizing landmark-based configurations, semi landmarks, and 3D morphometric techniques to capture morphological nuances that traditional linear measurements often overlook (Savriama, 2018; Harandi *et al.*, 2023). Methodologically, the integration of principal component analysis (PCA), thin-plate spline (TPS) interpolation, and allometric decomposition allows for the visualization of shape deformation and the identification of subtle morphological patterns (Klingenberg, 2016; Mitteroecker and Gunz, 2009). In plant sciences, these tools have proven highly effective for discriminating at the infraspecific level, including the analysis of leaf-shape mutants, floral symmetry, and fruit descriptors in diverse taxa such as *Arabidopsis* Heynh., olive cultivars, and eggplants (Backhaus *et al.*, 2010; Dehghan-Seresht *et al.*, 2025; Kaushik *et al.*, 2018; Chen *et al.*, 2018). By combining high-throughput landmarking with multivariate statistical integration, geometric morphometrics achieves high discrimination accuracy, making it indispensable for germplasm characterization and the assessment of biological diversity across multiple hierarchical levels (Manacorda and Asurmendi, 2018; Gardner *et al.*, 2016; Cope *et al.*, 2012).

To complement geometric morphometrics, fractal analysis offers a robust mathematical framework for quantifying the multiscale structural complexity of biological forms (Pham *et al.*, 2022). While traditional morphometrics focus on spatial arrangements of landmarks, the fractal dimension (FD) captures the self-similar properties of natural geometries (Mandelbrot, 1985), providing a critical metric for leaf margin dissection and shape irregularity (Borkowski, 1999). This approach serves as a non-redundant descriptor of plant development and structural evolution (Corbit and Garbary, 1995), allowing for finer taxonomic differentiation where geometric transformations might overlap (Bayirli *et al.*, 2015). By integrating FD as a supplementary parameter, researchers can achieve a more comprehensive characterization of leaf phenotype, bridging the gap between global shape variation and localized boundary complexity.

Advanced morphometric approaches, particularly multiscale Minkowski fractal dimensions, have demonstrated significant efficacy in leaf shape analysis within botanically complex taxa (Plotze *et al.*, 2005). By integrating volumetric fractal dimensions and texture-based descriptors, these methods capture intricate three-dimensional structural data and local surface coarseness that traditional techniques often overlook (Backes *et al.*, 2009; Florindo *et al.*, 2015). This computational framework provides a more nuanced characterization of plant morphology, ranging from the classification of highly variable species to the analysis of surface-level phenomena such as the Lotus Effect (Taraschi and Florindo, 2020; Yamamoto *et al.*, 2015). Consequently, fractal analysis offers a robust means of quantifying subtle biological variations, making it an essential tool for resolving diagnostic ambiguities in complex plant groups.

Beyond theoretical modeling, fractal dimension offers significant practical utility in environmental monitoring and automated plant identification. It facilitates accurate Leaf Area Index (LAI) retrieval by quantifying complex leaf distribution patterns within forest canopies (Jonckheere *et al.*, 2006). Furthermore, integrating fractal features into automated recognition systems (Du *et al.*, 2013; Bruno *et al.*, 2008) enhances the efficiency of species similarity analyses, providing a robust framework for distinguishing closely related taxa, such as those within the genus *Quercus* L. (Musarella *et al.*, 2018).

Historically, the classification of the *C. japonica* complex was guided by static morphological concepts that assumed leaf architecture reflected discrete, heritable divergences (Shan *et al.*, 1985). This early framework relied heavily on extreme phenotypes identified in herbarium-based studies, which established an artificial perception of discrete morphological clusters (Hussein *et al.*, 2021). However, the subsequent discovery of numerous intermediate phenotypes has fundamentally challenged the validity of the traditional three-form classification scheme (Pan *et al.*, 2005). These transitional specimens often defy clear categorization into established taxa (Wiens and Servedio, 2000), suggesting that previous taxonomic models failed to account for the continuous nature of morphological variation across different environments.

The application of geometric morphometrics is pivotal in resolving long-standing taxonomic ambiguities within complex plant lineages(Zapata & Jiménez, 2012). Unlike traditional classification methods, which often rely on subjective descriptive characters prone to observer bias and artificial subdivisions (Hara, 1962; Wilkin, 1999; Gage and Wilkin, 2008), this quantitative approach offers superior precision in capturing shape variation. Its primary advantage lies in the ability to distinguish subtle morphological discontinuities that traditional qualitative assessments frequently overlook. By providing a statistically rigorous framework, geometric morphometrics can effectively re-evaluate species boundaries—crucial for species like *C. japonica*—where traditional taxonomic markers fail to account for continuous variation (Di Pietro *et al.*, 2016; Menini Neto *et al.*, 2019). Furthermore, this method excels in clarifying taxonomically critical groups by demonstrating that characters previously regarded as discrete often exist as overlapping gradients (Liu and Hong, 2016), thereby ensuring a more objective delimitation of biological entities.

Geometric morphometrics addresses these taxonomic challenges by effectively decomposing shape and size, facilitating the identification of nuanced biological patterns through multivariate analysis (Heenan *et al.*, 2021). Its primary advantage lies in isolating taxonomically significant variation from confounding factors like allometry and asymmetrical development, which is particularly critical given the high phenotypic plasticity inherent in many plant lineages (Hodač *et al.*, 2023; Vogel Ely *et al.*, 2018). For complex groups such as the Orchidaceae, where linear metrics often lack the necessary resolution, these techniques provide the analytical depth required to refine species circumscriptions (Menini Neto *et al.*, 2019).

This study advances the analysis of biological forms by integrating geometric morphometrics with scale-invariant quantitative methods to resolve the morphological complexity of the *C. japonica* complex. Grounded in the developmental plasticity hypothesis, our framework posits that the taxon's pronounced variation in leaf architecture stems from a plastic "developmental toolkit" that facilitates phenotypic adaptation to environmental heterogeneity. By synthesizing these computational approaches, we move beyond unidimensional descriptions to systematically evaluate how such plastic responses shape morphological diversity within this complex. In this study, we revisit the taxonomy of *C. japonica* using a data-driven, multivariate approach. To address previous critiques regarding the subjective nature of visual classification, we apply unsupervised learning algorithms to a large morphometric dataset (n = 1,061). Our objectives are to: (1) determine whether the three traditionally recognized forms occupy distinct regions in morphospace; (2) test if the observed morphological variation is discrete or continuous using cluster validation metrics; and (3) quantify the relationship between leaf complexity and key geoclimatic variables to test the hypothesis of environmentally induced phenotypic plasticity.

Material and methods

Specimen Sampling and Herbarium Data

Specimens were collected from wild populations, with associated voucher specimens deposited at the herbarium of the Institute of Botany, Jiangsu Province and Chinese Academy of Sciences (NAS). Geographic coordinates were recorded in the field using GPS devices (WGS84) with an accuracy of ±5 m. We analyzed a total of 1,061 mature leaves of 1,052 samples from 72 wild populations across East Asia and 9 samples of the North American sister species *C. canadensis* (Appendix S3: S1_Dataset_Populations and S2_Dataset_Individuals). Sampling locations spanned a latitudinal range from 22.4°N to 40.9°N and an altitudinal gradient from 15 m s.l.m to 2005 m s.l.m across China, Korea, and Japan (Appendix S1: Figure 1).

Digital Image Acquisition and Preprocessing

Leaf images were captured using a Canon EOS R5 camera (Canon Inc., Japan) equipped with an RF 100mm f/2.8 macro lens (Canon). Lighting was standardized using a ring-flash (NEEWER TT560) at 45° angle to minimize shadow artifacts. A color reference standard was placed in each image frame to enable post-hoc color normalization. All images were scanned at 1024 × 1024 dpi (equivalent to ~25 μm pixel resolution) and saved as uncompressed TIFF files. Image quality was verified through histogram analysis to ensure adequate contrast and minimal blown-out highlights. Any image with perceptible damage, insect herbivory, or fungal infection was excluded from analysis. To ensure high-fidelity outline capture, a custom multi-scale gradient-based edge detection algorithm was implemented in MATLAB® R2013a (The MathWorks, Natick, MA, USA) (Appendix S3: Algorithm presents details for leaf outline). Leaf area,

minimum rectangle, perimeter, and the length of vertical and horizontal axes were quantified with ImageJ v. 1.53k (Appendix S1: Figure 2).

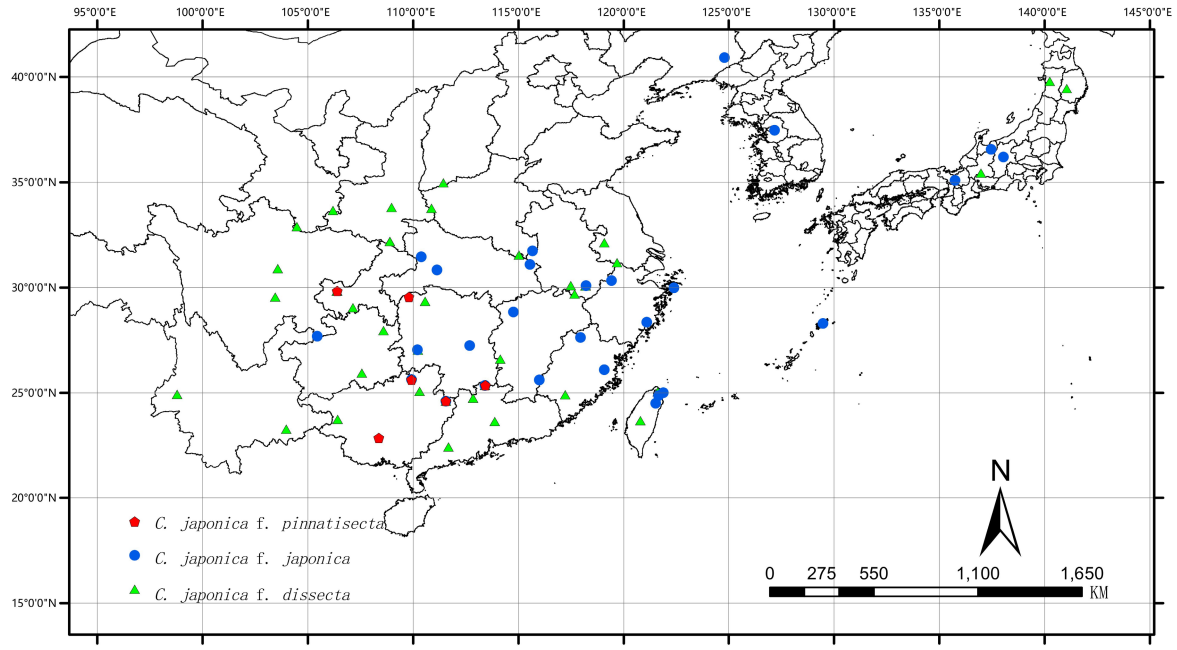

FIGURE 1. Geographic distribution of *Cryptotaenia japonica* and sampling locations across East Asia (The map was produced by ArgGIS V.2022 Q2 Release).

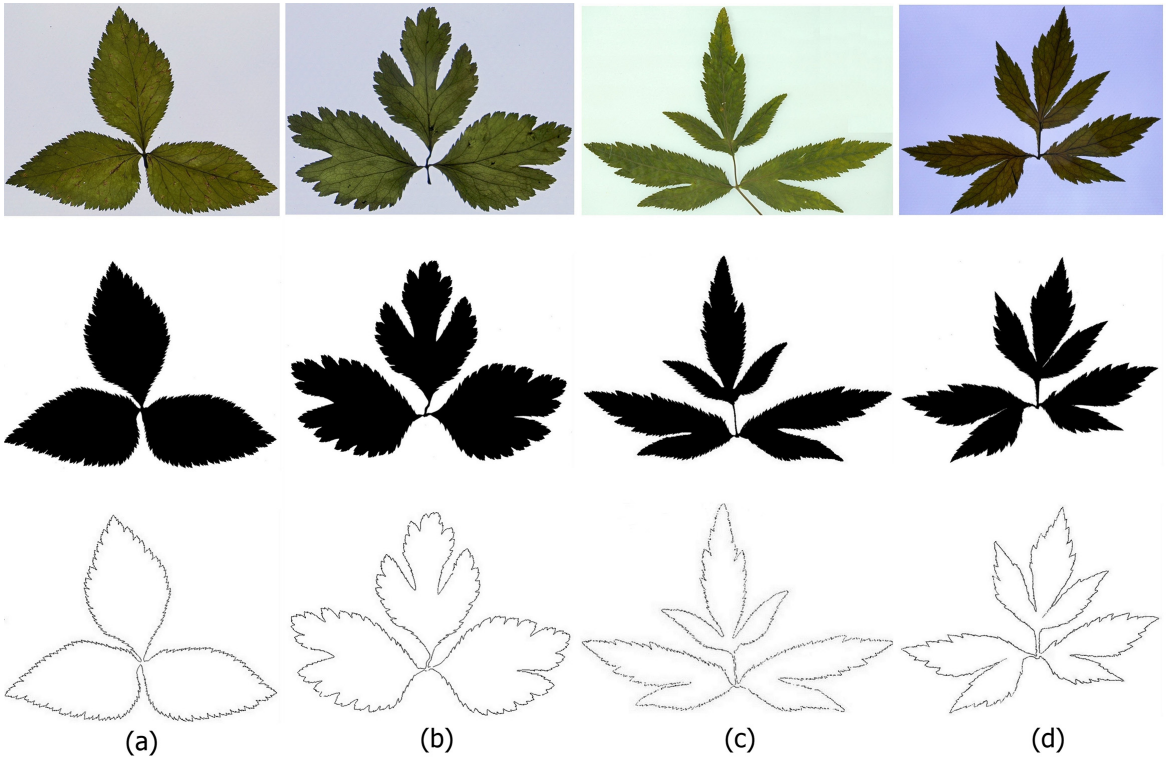

FIGURE 2. Examples of digital images (top), images for edge recognition (middle), and outlines for fractal dimension (bottom). (a) *C. japonica* f. *japonica*; Xiushuixian, Jiangxi, China, (b) *C. japonica* f. *dissecta*; Jinyunshan, Chongqing, China; (c) *C. japonica* f. *pinnatisecta*; Sangzhixian, Hunan, China; (d) *C. canadensis*, Newark, Ohio, USA.

Morphometric Parameter Selection and Justification

Four key morphometric parameters were extracted to define the global morphospace of leaf shape:

Fractal Dimension (FD): Calculated via the box-counting method using Fractalyse software (version 2.4). FD provides a scale-invariant measure of leaf margin complexity, with values ranging from 1.0 to 2.0.

Leaf Coefficient (LC): Defined as 16 × (Area / Perimeter²), this index quantifies leaf compactness or roundness in a standardized manner.

Rectangularity (RE): The ratio of the leaf area to the area of its minimum bounding rectangle.

Aspect Ratio (VH): The ratio of the vertical axis length to the horizontal axis length of the leaf's bounding box.

Fractal Dimension was prioritized as the primary metric for leaf complexity because it is invariant to scale and rotation, effectively capturing the hierarchical, self-similar properties of leaf-margin architecture. Rectangularity and Aspect Ratio were retained to describe overall lamina shape beyond mere complexity, while Leaf Coefficient quantifies global compactness. Together, these four parameters represent an interpretable and non-redundant subset capable of capturing the essential dimensions of the leaf morphospace.

Multivariate Statistical Analysis

Principal Component Analysis (PCA)

Morphometric heterogeneity among the four predefined taxonomic units (f. *japonica*, f. *dissecta*, f. *pinnatisecta*, and *C. canadensis*) was first evaluated using the non-parametric Kruskal-Wallis omnibus test, followed by Bonferroni-corrected Dunn's post-hoc pairwise comparisons. Individual-level data (n = 1,061) were log-transformed where necessary to meet the assumption of symmetry. The Kruskal-Wallis test was applied separately to each of the four leaf-shape indices (Fractal Dimension, Rectangularity, Leaf Coefficient, and Aspect Ratio). Pairwise differences were examined only when the omnibus test yielded a significance of $P< 0.05$. The significance threshold for all post-hoc comparisons was adjusted using the Bonferroni method to control the family-wise error rate at $\alpha = 0.05$. Effect sizes are reported as *H*-statistics. All tests were two-tailed.

Principal Component Analysis was performed on the correlation matrix using the FactoMineR in R (version 4.5.0; R Core Team, 2025). A scree plot indicated that the first three principal components cumulatively explained >85% of the total variance; however, only PC1 and PC2 (together explaining 68.63% of the variance) were retained for downstream analyses based on the Kaiser criterion (eigenvalue > 1.0). To assess the robustness of the PCA ordination, we performed 1,000 bootstrap replicates of the correlation matrix and plotted 95% confidence ellipses around the group centroids.

Hierarchical Clustering and Silhouette Validation

To quantify the stability of any recovered clusters, we implemented a "Full-Pipeline Bootstrap" procedure with 1,000 iterations to calculate the 95% confidence interval (CI) for the Adjusted Rand Index (ARI). Each iteration rigorously executed the following steps:

1. **Resampling:** A bootstrap sample was drawn with replacement from the standardized morphometric matrix.
2. **Recalculation:** A new Euclidean distance matrix was computed from the resampled data, and a new dendrogram was constructed via Ward's linkage method for each bootstrap sample.
3. **Validation:** The resulting cluster assignments (at $k= 3$ clusters) were compared against the traditional taxonomic labels to calculate the ARI.

Recalculating both the distance matrix and the dendrogram in every iteration is methodologically essential. It accounts for the stochasticity in cluster topology and the sensitivity of Ward's algorithm to sample composition, unlike simply reshuffling labels on a static tree.

Cluster stability was further assessed using the gap statistic, which compares the within-cluster dispersion to that expected under a null distribution. The optimal number of clusters (k) was identified as the value that maximized the gap statistic. Additionally, we computed the Dunn Index—defined as the ratio of the minimum inter-cluster distance to the maximum intra-cluster distance—for $k= 1$ to $k= 8$. This analysis confirmed that a two-cluster solution ($k= 2$, Dunn Index = 0.205) yielded superior separation compared to the traditional three-cluster model ($k= 3$, Dunn Index = 0.193).

Generalized Additive Models (GAM)

The relationship between the morphological complexity axis (represented by PC1 scores) and altitude was modeled using a Generalized Additive Model (GAM) with thin-plate regression splines (basis bs = "tp"). Parameters were estimated via Restricted Maximum Likelihood (REML). This non-parametric approach was selected to accommodate

the potential non-linear "reaction norms" inherent in phenotypic plasticity, which traditional parametric models often fail to capture. The model structure is expressed as: $g(E[Y]) = \beta_0 + f(\text{Altitude}) + \varepsilon$, where $Y$is the PC1 score and $f$is a non-parametric smoothing function. The basis dimension for the smooth term was set to k= 10, providing sufficient degrees of freedom to capture a potential sigmoidal transition along the altitudinal cline while maintaining model parsimony.

Model diagnostics were conducted using the fitted() and residuals() functions. Concurvity (the GAM analogue of multicollinearity) was assessed between the altitude and latitude predictors; the low estimate (0.14) indicated that these variables contributed largely independent information to the model. Partial autocorrelation plots of the model residuals revealed no significant temporal or spatial clustering (maximum autocorrelation at lag-1: r= 0.082, n.s.), validating the assumption of independent observations.

Reproducibility and Code Availability

All analyses and figure generation were performed using R version 4.5.0 (R Core Team, 2025). Key packages used included tidyverse 2.0.0, readxl 1.4.5, dplyr 1.1.4 for data manipulation, ggplot2 4.0.1 and related packages (ggpubr 0.6.2, factoextra 1.0.7, cowplot 1.2.0, ggrepel 0.9.6, scales 1.4.0) for visualization, and rstatix 0.7.3, FactoMineR 2.13, agricolae 1.3-7, cluster 2.1.8.1, mclust 6.1.2, and mgcv 1.9-1 for statistical analyses.

The raw morphological and environmental data, along with all R scripts used for data preprocessing, statistical analyses, and figure generation, have been deposited in Figshare and are publicly available under the DOI: https://doi.org/10.6084/m9.figshare.31839943.

All figures were generated programmatically using the provided scripts. No manual image editing or post-hoc modifications were performed. Detailed technical specifications regarding algorithm execution and visualization workflows are provided in Appendix S3.

Results

Quantitative Morphological Divergence

Non-parametric Kruskal-Wallis tests revealed highly significant morphological heterogeneity across all four measured leaf traits among the predefined groups (P< 0.001; see Appendix S2: Table 1, Appendix S1: Figure 3, and Appendix S2: Table 2 for details). Fractal Dimension (FD) emerged as the most diagnostic descriptor of leaf complexity, exhibiting the greatest discriminatory power among all variables tested (H= 275.8, df= 3, P< 0.001). The forms f. *dissecta* and f. *pinnatisecta* displayed markedly elevated FD values compared to the typical f. *japonica*, reflecting their more intricate venation and lobing patterns. Rectangularity (RE) also demonstrated robust inter-group differentiation (H= 57.7, P< 0.001), indicating substantial variation in leaf shape compactness. Bonferroni-corrected Dunn's post-hoc comparisons confirmed that *C. canadensis* remains morphologically distinct from the deeply-lobed East Asian forms (f. *dissecta* and f. *pinnatisecta*). The North American species is characterized by a significantly more compact leaf architecture, as evidenced by its consistently higher RE values and correspondingly lower FD values (Appendix S2: Table 1). These morphometric patterns underscore fundamental architectural differences distinguishing the North American species from its phylogenetically related Asian congeners.

TABLE 1. Summary statistics of leaf morphological traits in *Cryptotaenia japonica* forms and *C. canadensis.*

Trait	f. *japonica* (*N*=369)	f. *dissecta* (*N*=657)	f. *pinnatisecta* (*N*=26)	*C. canadensis* (*N*=9)	Overall CV (%)
FD	1.258 ± 0.052 a	1.319 ± 0.055 b	1.406 ± 0.120 c	1.403 ± 0.092 c	5.10
RE	0.420 ± 0.038 a	0.416 ± 0.035 a	0.346 ± 0.068 b	0.322 ± 0.022 c	9.55
LC	0.082 ± 0.021 a	0.066 ± 0.018 b	0.061 ± 0.014 b	0.042 ± 0.007 c	29.09
VH	0.756 ± 0.049 a	0.773 ± 0.059 b	0.796 ± 0.057 b	0.782 ± 0.048 ab	7.37

Note: Values represent mean ± standard deviation. Different superscript letters within a row indicate statistically significant differences based on Bonferroni-corrected Dunn's post-hoc tests (P< 0.05). CV, coefficient of variation; FD, Fractal Dimension; RE, Rectangularity; LC, Leaf Coefficient; VH, vertical to horizontal axis ratio. *N*, sample size.

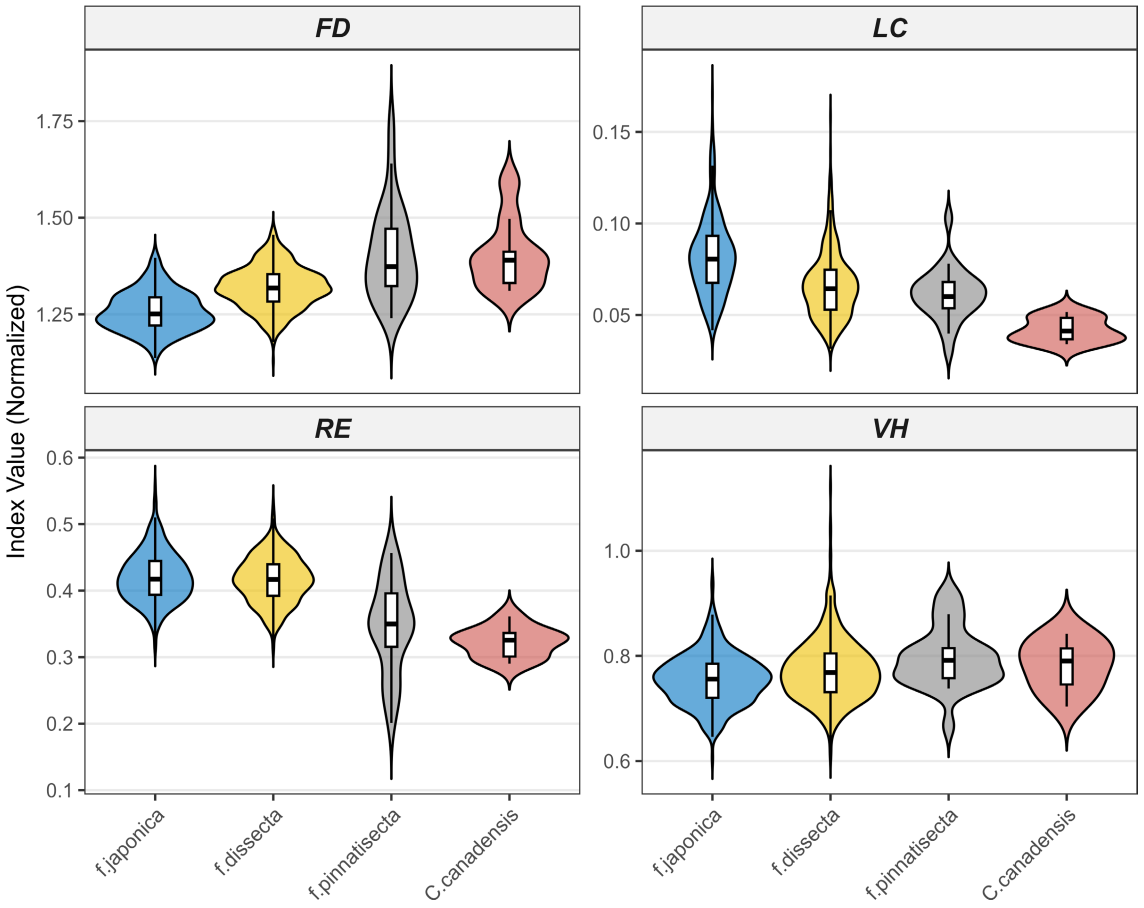

FIGURE 3. Morphometric descriptors across *Cryptotaenia japonica* forms and *C. canadensis*. Violin plots with embedded boxplots (horizontal lines = quartiles, black dot = median) and jittered data points show the probability density and distribution of four morphometric parameters (*FD*, *RE*, *LC*, *VH*).

TABLE 2. Results of Kruskal-Wallis tests and post-hoc comparisons for leaf morphological metrics among *Cryptotaenia*taxa.

Metric	*H* statistic	*df*	*p*-value	Post-hoc Significance (Dunn's)
FD	275.8	3	< 0.001***	D, P > J > C
RE	57.7	3	< 0.001***	C, J > P > D
LC	34.2	3	< 0.001***	D > J, P > C
VH	28.5	3	< 0.001***	D > P, J > C

Note: Significance level: ***P*< 0.001**. Post-hoc groups are ordered by descending mean rank and summarized as: J, f. *japonica*; D, f. *dissecta*; P, f. *pinnatisecta*; C, *C. canadensis*. The ">" symbol indicates a statistically significant difference in the trait value (e.g., for FD: D, P > J > C means f. *dissecta*and f. *pinnatisecta* have significantly higher FD than f. *japonica*, which in turn has higher FD than *C. canadensis*).

Multivariate Morphospace and Component Loadings

Principal Component Analysis (PCA) elucidated the primary dimensions of morphological variation, with the first two principal components (PCs) cumulatively accounting for 68.63% of the total variance (Appendix S1: Figure 4). PC1, which explained 40.6% of the variance, served as a robust proxy for leaf margin complexity. One-way ANOVA revealed highly significant differences in PC1 scores among the examined taxa (F[3, 1057] = 100.4, P< 0.001). Post-hoc comparisons using Tukey's HSD test delineated the four forms into three statistically distinct morphological cohorts (Appendix S1: Figure 5): **Group a:** *C. canadensis*, exhibiting the highest morphological complexity (i.e., highest mean PC1 score). **Group b:** f. *pinnatisecta*, occupying an intermediate position in morphospace. **Group c:** f. *dissecta*and f. *japonica*; although minor differences in mean values were detected, these two forms demonstrated substantial morphological overlap along the PC1 axis and were statistically indistinguishable.

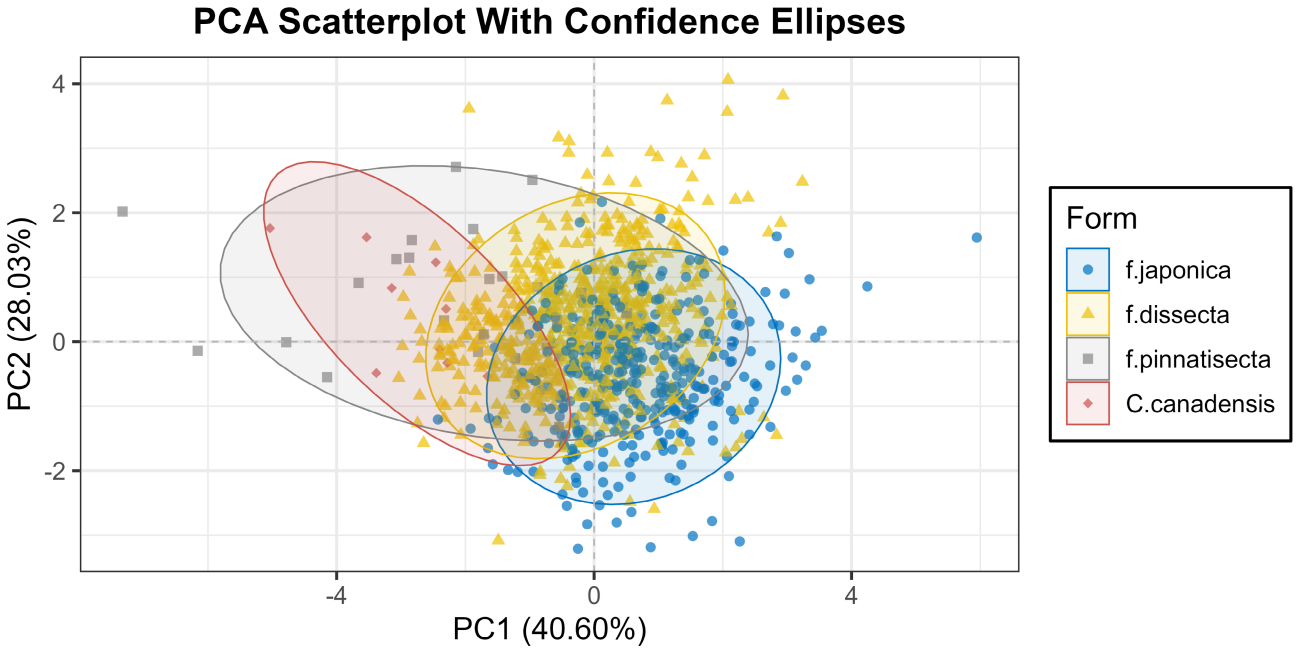

FIGURE 4. Principal Component Analysis (PCA) of morphometric space. Scatterplot showing PC1 (40.60% variance) vs PC2 (28.03% variance) with 95% confidence ellipses for each taxon. Colored vectors represent variable loadings (FD, RE, LC, VH), indicating the contribution of each morphometric descriptor to the principal components.

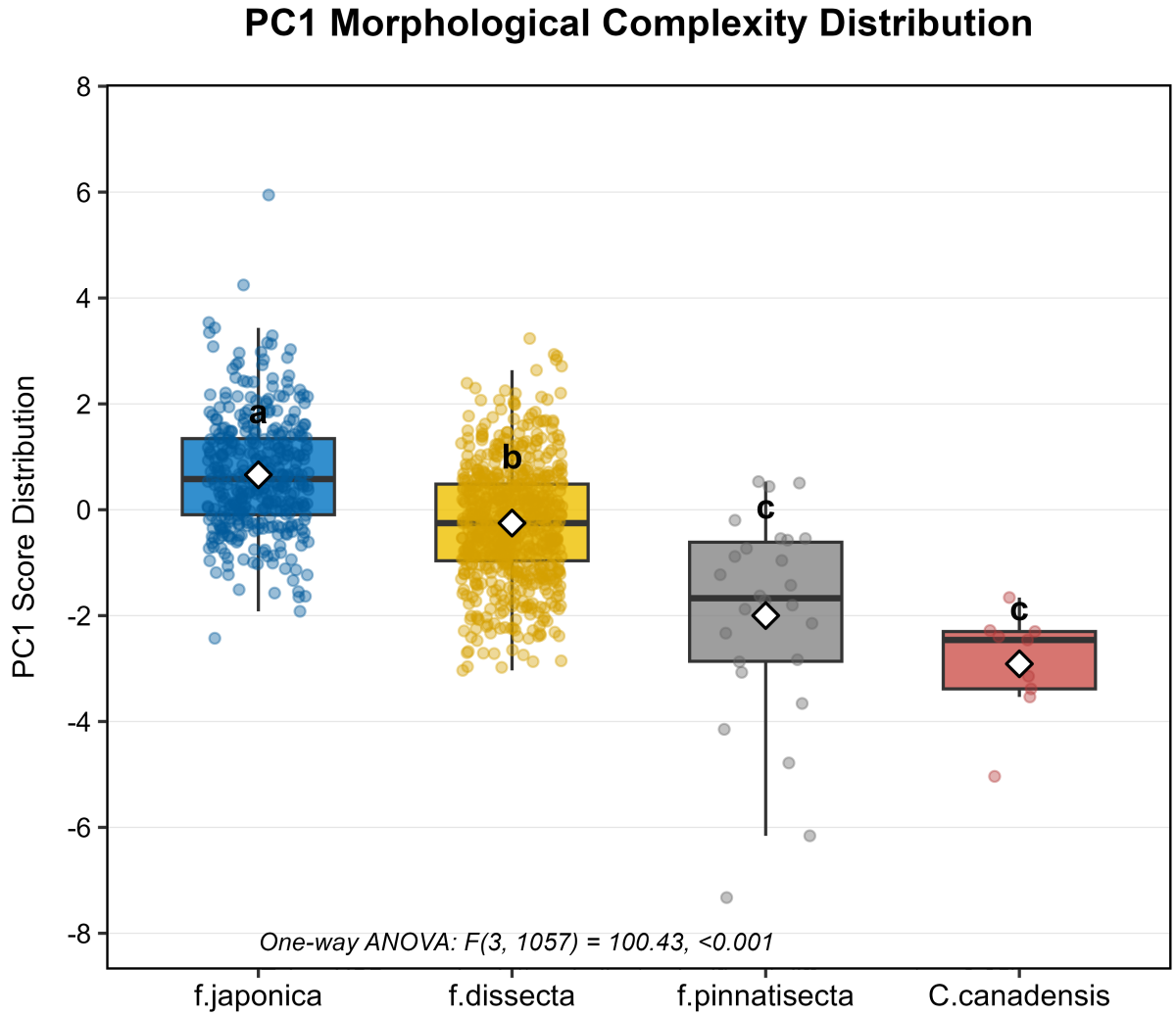

FIGURE 5. Box plot of PC1 scores for the four taxonomic units. Different letters (a, b, c) above boxes indicate statistically significant groupings based on Tukey's HSD test ($P < 0.05$).

These results demonstrate a clear gradient of increasing morphological complexity from *C. japonica* f. *japonica* to *C. canadensis*. The position of f. *dissecta* underscores its role as a morphological transition, or "bridge," between f. *japonica* and f. *pinnatisecta* within the observed phenotypic continuum.

Taxonomic Re-evaluation Metrics

Determination of the optimal number of clusters via Average Silhouette Width (ASW) analysis revealed a definitive peak at k=2 (ASW = 0.205), identifying this as the statistically optimal partition for the dataset (Appendix S1: Figure 6). In contrast, the ASW for the traditionally hypothesized three-cluster configuration (k= 3) decreased to 0.193, indicating a substantially weaker and less justified partition structure. This lack of robust clustering for the three-form model was further corroborated by the Adjusted Rand Index (ARI). The ARI between the unsupervised clustering result at k=3 and the traditional taxonomic labels was 0.088 (95% CI: 0.016–0.153). Given that an ARI of 1.0 denotes perfect concordance and 0 indicates a match equivalent to random chance, a coefficient of 0.088 provides rigorous statistical grounds to reject the conventional tripartite classification schema.

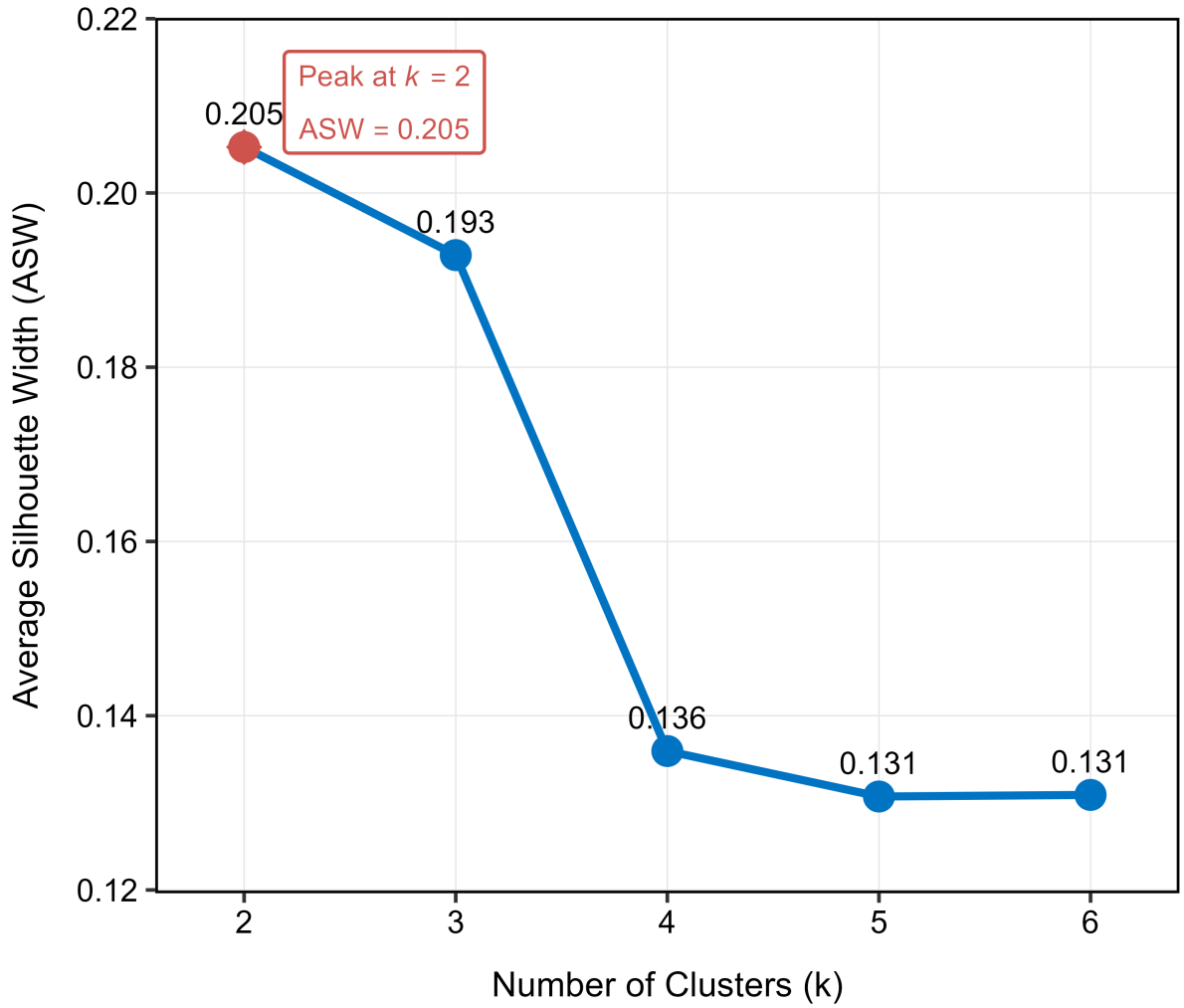

FIGURE 6. Silhouette analysis for determining the optimal number of clusters. The peak at k=2 (ASW = 0.205) indicates poor correspondence between unsupervised clustering and the traditional three-form taxonomic assignment (k=3), statistically refuting the validity of the latter.

Threshold Detection via Generalized Additive Model Analysis

To characterize the relationship between leaf complexity (represented by PC1 scores) and elevation, we employed a Generalized Additive Model (GAM) with thin-plate regression splines. This non-linear modeling approach allows for the detection of complex phenotypic responses across environmental gradients. The REML-optimized model revealed a statistically significant altitudinal trend (adjusted R^2 = 0.0559, P< 0.001). Furthermore, a segmented regression analysis optimized by the Akaike Information Criterion (AIC) identified an inflection point near 850 m s.l.m, above

which leaf complexity tends to increase more steeply, a pattern consistent with the widely recognized hypothesis that increased leaf dissection enhances convective heat dissipation under high-altitude conditions (ZHU *et al.*, 2018) (Appendix S1: Figure 7). Although the model explains only a small fraction of the total variance, the significant non-linear pattern is consistent with the hypothesis that leaf morphology responds to altitudinal environmental gradients; the remaining variance likely reflects unmeasured local microhabitat factors or stochastic developmental variation. It should be noted that altitude is used here as a coarse proxy for composite environmental variables (e.g., temperature, UV radiation, humidity); direct microhabitat measurements are needed to better quantify the environmental contribution to leaf morphological variation.

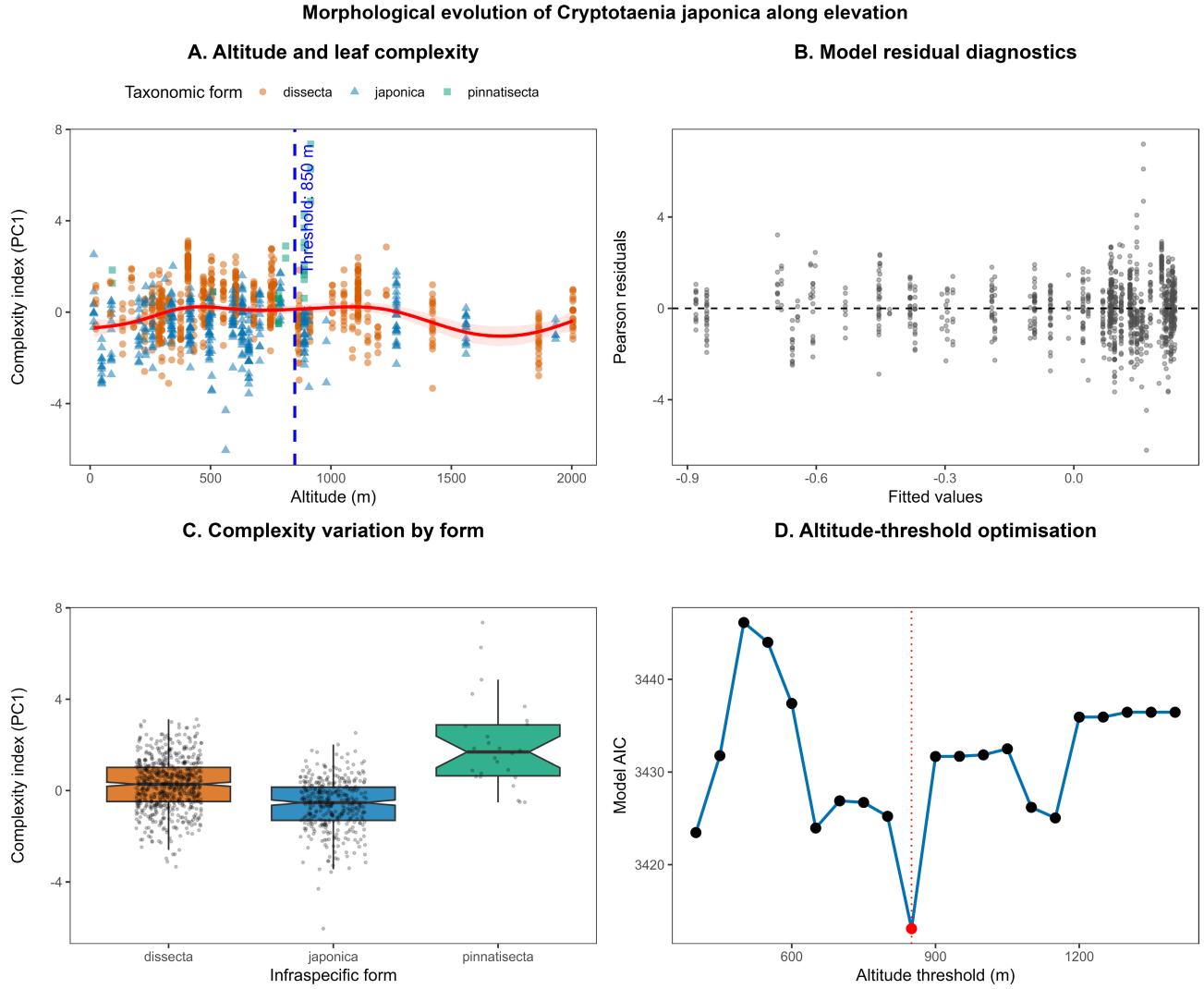

FIGURE 7. Morphological evolution of *Cryptotaenia japonica* along an elevational gradient. (A) The non-linear relationship (altitudinal cline) between leaf complexity (PC1) and elevation, as fitted by the Generalized Additive Model (GAM), with a shaded area representing the 95% confidence interval. The vertical dashed line indicates an inflection point around 850 m s.l.m identified by segmented regression. (B) Diagnostic plots for model validation, showing the uniform distribution of Pearson residuals against fitted values. (C) Distribution of sampling effort across the traditional infraspecific taxa, illustrating the quantitative representation of the morphological *continuum* (Total N= 1,052). (D) Threshold optimization based on the Akaike Information Criterion (AIC). The overall GAM (adjusted R^2 = 0.0559, P< 0.001) indicates a modest but statistically significant altitudinal trend in leaf complexity.

Discussion

Continuous Morphological Variation and the Limitations of Discrete Taxonomic Categorization

Our quantitative findings provide evidence against the validity of the traditional tripartite classification of the *C. japonica* complex, as the morphological boundaries previously used to delineate these taxa do not withstand rigorous

statistical scrutiny. The low Adjusted Rand Index (ARI = 0.088) and its confidence interval indicate that the recovered clustering pattern shows no meaningful correspondence with the current infraspecific designations. This mirrors the taxonomic instability observed in other plant groups where diagnostic characters exhibit extensive overlap (Vogel Ely *et al.*, 2018). Principal Component Analysis further demonstrates that f. *dissecta* occupies an intermediate morphological position, bridging f. *japonica* and f. *pinnatisecta* rather than forming a discrete cluster. This lack of clear differentiation suggests that the diagnostic value of the historical characters has been overestimated, a phenomenon also documented in revisions of other wide-ranging species complexes (Liu and Hong, 2016). Such phenotypic continuity underscores the necessity of moving from subjective, qualitative assessments towards objective, data-driven methods for resolving species circumscriptions (Hodač *et al.*, 2023).

The purported distinction between f. *japonica* and f. *dissecta* is further challenged by the likelihood of herbarium sampling bias, wherein historical classifications were often based on conspicuous, extreme morphotypes rather than the full spectrum of continuous variation present in natural populations. As we acknowledge in the "Limitations and Future Perspectives" section, our own herbarium-sourced dataset may be subject to analogous collection biases, and this warrants caution when interpreting the apparent distinctness of morphological clusters. To mitigate this bias, we employed a robust sampling strategy across extensive altitudinal gradients. Our analysis reveals significant morphological overlap between f. *japonica* and f. *dissecta* in leaf architecture, a pattern congruent with findings in other complex taxa where putative diagnostic characters fail to maintain stability under quantitative evaluation (Liu and Hong, 2016). Such variability necessitates caution in defining taxa along environmental gradients to avoid taxonomic artifacts derived from incomplete sampling (Menini Neto *et al.*, 2019). Given that our specimens do not cluster according to the traditional infraspecific categories—a situation analogous to that in the *Hypericum rigidum* complex where subspecies show broad morphological and geographical overlap (Vogel Ely *et al.*, 2018)—the diagnostic boundaries between these forms are effectively invalidated. The evidence suggests that f. *japonica* and f. *dissecta* represent points along a continuous spectrum of phenotypic plasticity rather than distinct evolutionary lineages. This conclusion necessitates a formal taxonomic revision to synonymize these overlapping entities.

Methodological Foundations and Implications of Unsupervised Multivariate Morphometrics

This study exemplifies a significant methodological advance in plant systematics: the transition from traditional, morphology-based classification to a data-driven paradigm employing unsupervised learning. Conventional taxonomic practice often relies on an *a priori* categorical framework, where specimens are deductively assigned to predefined taxa based on a set of presumed discrete diagnostic traits. This approach is inherently susceptible to observer bias and subjective interpretation, particularly when dealing with the high-dimensional morphological variation characteristic of species complexes. By prioritizing expert-led qualitative assessment over quantitative pattern recognition, traditional methods may inadvertently obscure subtle yet biologically meaningful structural continuities.

Unsupervised learning algorithms provide a robust alternative by inductively exploring biological data, allowing the intrinsic structure of the dataset to define the number and composition of clusters without the constraint of prior taxonomic hypotheses. A key strength of these methods is their capacity to uncover latent patterns in phenotypic space through objective statistical criteria. Specifically, the Silhouette Analysis employed here offers a rigorous, assumption-free measure of clustering quality, as it does not presuppose the number of clusters or their distribution within the morphospace. By integrating multiple validation metrics—including the Average Silhouette Width (ASW), Adjusted Rand Index (ARI), and the Dunn Index—our analytical framework generates convergent evidence for the stability of identified partitions. In the present case, the consistent indication across these metrics that $k = 2$ represents the optimal partition provides strong, multi-faceted support for this conclusion.

Such paradigm shifts in analytical approach are increasingly documented across various plant lineages with contentious taxonomic boundaries. Recent systematic studies of complexes such as *Ranunculus auricomus* (Hodač *et al.*, 2024) and in the genus *Quercus* (Qi *et al.*, 2024) demonstrate that quantitative morphological analyses often reveal more fluid boundaries than those suggested by classical descriptions. These findings collectively show that unsupervised methods can resolve long-standing taxonomic ambiguities historically perpetuated by high phenotypic plasticity and inter-specific morphological overlap.

In conclusion, while classical infraspecific classifications retain conceptual utility for field identification and general botanical communication, our results underscore that they frequently lack robust statistical justification. These traditional categories may impose artificially discrete boundaries upon natural populations characterized by continuous variation. By adopting unsupervised learning, we advance towards a more objective systematic framework that better reflects biological reality, acknowledging that human-defined subspecies labels often fail to capture the complex, underlying structure of plant diversity.

Comparative Systematic Analysis of *Cryptotaenia* within the Apiaceae Family

The Apiaceae family is characterized by significant morphological plasticity, which often hinders consistent taxonomic identification. Within the family, the genus *Chaerophyllum* L. exemplifies a complex group where traditional circumscriptions based on fruit and vegetative traits frequently overlap with related taxa. Such pronounced morphological variability is evident in species like *Conium maculatum* L. and *Oenanthe aquatica* (L.) Poir., which exhibit substantial within-population variation in response to local ecological conditions (Downie *et al.*, 2000). This phenotypic diversity is largely driven by environmental factors such as soil moisture, light availability, and nutrient gradients, leading to heterogeneous forms that can obscure phylogenetic boundaries. Previous studies have highlighted the limitations of relying solely on qualitative descriptions for these groups; for instance, phylogenetic research on related tribes has noted how morphological convergence complicates clear taxonomic delineation (Downie *et al.*, 2008). Furthermore, investigations into reproductive and architectural traits of the Apiaceae underscore the necessity of integrating multiple character sets to resolve evolutionary relationships. These collective findings highlight the inadequacy of traditional descriptive methods and motivate the application of rigorous quantitative morphometric analyses to provide a more objective framework for understanding the structural diversity and taxonomic status of *Chaerophyllum* and its relatives.

A comparative analysis of related Apiaceae genera, including *Chaerophyllum*, *Conium*, and *Oenanthe*, underscores the necessity of applying quantitative geometric morphometrics to reassess systematic relationships and taxonomic boundaries within the family. This approach is particularly warranted given the morphometric insights gained from studies on *Daucus* L. (Arbizu *et al.*, 2014) and the significant homoplasy (similarity due to convergent evolution rather than common descent) identified in fruit characters across Apiaceae (Banasiak *et al.*, 2016).

Evolutionary Strategies: Plasticity vs. Canalization

The divergence between the East Asian *Cryptotaenia japonica* and its North American sister species, *C. canadensis*, reflects a profound shift in evolutionary strategy following their intercontinental isolation approximately 15–20 million years ago (Spalik and Downie, 2007). This vicariance event initiated distinct adaptive trajectories shaped by the contrasting biogeographic and climatic histories of their respective regions.

In East Asia, *C. japonica* exhibits an evolutionary strategy centered on high phenotypic plasticity. This adaptive flexibility is evidenced by the significant variation in leaf morphology (captured by FD and other shape indices) across the altitudinal gradients (Debat and David, 2001) documented in this study. Within the topographically complex and heterogeneous montane environments of East Asia, such plasticity serves as a critical mechanism, enabling populations to fine-tune vegetative structures in response to local environmental cues, thereby maximizing fitness across diverse microhabitats.

In stark contrast, the North American lineage, *C. canadensis*, exhibits a comparatively stable leaf phenotype in our dataset, a pattern suggestive of evolutionary canalization (Siegal and Bergman, 2002). Our quantitative morphological analyses reveal that *C. canadensis* maintains a highly stable leaf phenotype despite environmental fluctuations, characterized by significantly lower coefficients of variation (CV%) and more restricted ranges in FD and other shape indices compared to its East Asian congener (see Appendix S2: Table 1). This trait uniformity suggests a history of genetic assimilation, whereby once-plastic developmental responses have become genetically fixed through selection for a robust, reliable phenotype (Velotta *et al.*, 2018). This stabilization likely resulted from historical population bottlenecks during late Tertiary climatic shifts or repeated Pleistocene glacial cycles in North America, which reduced genetic diversity and favored canalized survival strategies within more stable or homogeneous refugia. Thus, while the East Asian lineage has thrived through adaptive versatility, the North American lineage exhibits lower phenotypic variance, suggesting a possible shift in evolutionary strategy, while the East Asian lineage maintains high morphological plasticity, representing two fundamentally different responses to long-term continental isolation, although our limited North American sample (n=9) warrants cautious interpretation.

Phenotypic Plasticity and Thermal Regulation

The increase in leaf architectural complexity along elevation gradients serves as a critical adaptive solution to the thermodynamic challenges posed by high-altitude environments. Empirical studies on phenotypic plasticity in extreme environments suggest a significant positive correlation between altitude and the degree of leaf dissection (Chevin and Hoffmann, 2017). This phenomenon is largely governed by the "dissection-efficiency hypothesis," which posits that

deeply lobed or serrated margins reduce the thickness of the boundary layer of still air surrounding the leaf, thereby enhancing convective heat dissipation (ZHU *et al.*, 2018). Such morphological adjustments are crucial for preventing solar-induced thermal damage in the rarefied atmospheres of high elevations. This adaptive capacity aligns with the concept of a reaction norm, where a single genotype produces a range of phenotypes in response to environmental variation, as a fundamental mechanism for maintaining physiological homeostasis under fluctuating thermal regimes (Pigliucci *et al.*, 2006).

Furthermore, altitudinal morphological differentiation often involves genetic accommodations that optimize physiological trade-offs. Under the extreme conditions of high elevations, plants frequently exhibit a reduced Specific Leaf Area (SLA) coupled with increased leaf margin complexity. This suite of traits represents a strategic balance between maximizing carbon gain and minimizing water loss through transpirational cooling (Bakhtiari *et al.*, 2019). The ecological significance of these findings underscores that leaf form is a key functional trait influencing plant survival by modulating the energy balance of the leaf surface (phyllosphere), reflecting the complex interplay between environmental selection pressures and evolutionary constraints (Valladares *et al.*, 2007).

Taxonomic implications

The taxonomic status of *Cryptotaenia japonica* and its infraspecific forms requires reassessment in light of the quantitative evidence presented here. Our analysis yields a low Adjusted Rand Index (ARI = 0.088 for k= 3) and identifies k= 2 as the optimal clustering solution, which statistically refutes the validity of the recognized distinctions between f. *dissecta* and f. *pinnatisecta*. These findings suggest that the morphological variation traditionally used to delineate these taxa does not correspond to discrete genetic or evolutionary lineages. Instead, the observed diversity is consistent with a single, hyper-variable species, with f. *dissecta* and f. *pinnatisecta* representing altitudinal morphological variants rather than discrete genetic lineages.

This interpretation is strongly supported by the geometric continuity observed in leaf shape transitions, which indicates a phenotypic spectrum rather than discrete character states. Furthermore, the absence of clear population structure at the infraspecific level is consistent with high levels of gene flow and low genetic differentiation, as characterized in recent genomic studies of the species (Wu *et al.*, 2024). While *C. canadensis* exhibits a canalized divergence from the ancestral morphology (Hawkins *et al.*, 2005), *C. japonica* appears to maintain a high degree of phenotypic plasticity.

Consequently, the data-driven evidence suggests that the infraspecific ranks defined by traditional treatments (Liou, 1990) should be formally reconsidered. By treating f. *dissecta* and f. *pinnatisecta* as synonyms under *C. japonica*, we more accurately reflect the plastic nature of this species across its broad ecological range in East Asia. Recognizing *C. japonica* as a unified, phenotypically variable entity provides a better representation of its evolutionary history and biological reality, moving away from an artificial partitioning of continuous morphological variation. We therefore propose the abandonment of these formal infraspecific names in favor of a single, polymorphic species description, pending validation through independent genomic and common-garden data.

Implications for Conservation and Applied Botany

Recognizing *C. japonica* as a single, phenotypically plastic species underscores the importance of preserving environmental heterogeneity—particularly along altitudinal gradients—to maintain its adaptive potential. The concept of altitude-dependent phenotypic variation suggests that habitat fragmentation poses a significant threat by disrupting the continuous morphological cline (de Villemereuil *et al.*, 2018). Conservation strategies should therefore prioritize maintaining or restoring habitat connectivity across elevations to prevent the erosion of this adaptive diversity and ensure the long-term resilience of populations in the face of climatic change.

Furthermore, the extensive phenotypic plasticity identified in *C. japonica* necessitates a re-evaluation of horticultural germplasm management practices. As observed in other alpine herbs, leaf trait variation often mediates critical environmental adaptations (Geange *et al.*, 2017). Consequently, for cultivated forms, breeding and selection strategies should emphasize physiological performance and stability rather than maintaining leaf-shape-based cultivar distinctions (e.g., “dissecta”), as these phenotypes are largely environmentally induced rather than genetically fixed. This understanding can significantly streamline crop management protocols.

Finally, our demonstration of a data-driven, unsupervised morphometric workflow establishes a robust methodological framework for resolving similar taxonomic ambiguities. This multivariate approach aligns with the

principles of integrative taxonomy, facilitating species delimitation based on multiple, objectively defined characters (Padial *et al.*, 2010). Such a workflow has broad applicability across other economically or ecologically important plant groups where high phenotypic plasticity and complex nomenclature frequently obscure true evolutionary boundaries.

Limitations and Future Perspectives

Sampling Constraints and Statistical Considerations

Several methodological caveats warrant acknowledgment. First, regarding sample size imbalance, *C. japonica* f. *pinnatisecta* (*n*= 26) and *C. canadensis* (*n*= 9) are underrepresented compared to f. *dissecta* (*n*= 657) and f. *japonica*. Although the unsupervised algorithms employed are generally robust to unequal group sizes, more extensive sampling of these rare morphotypes would enhance the statistical power and generalizability of our inferences. Second, concerning statistical assumptions, some multivariate methods assume approximate multivariate normality. While we applied log-transformations and used non-parametric tests (Kruskal-Wallis) where appropriate, and Shapiro-Wilk tests on the first two principal components indicated no severe deviation from normality ($P > 0.05$), this assumption should be verified with larger, more balanced samples in future studies. Third, our environmental models used altitude and geographic coordinates as proxy variables. Incorporating direct, fine-scale measurements of microhabitat factors—such as solar radiation, soil moisture, and temperature regimes—would facilitate a more mechanistic understanding of the environment-morphology relationship.

Geographical Constraints and Generalizability

While this study extensively sampled East Asian populations of *C. japonica*, the analysis of *C. canadensis* was limited to nine North American specimens. To rigorously test the canalization hypothesis for this species, future sampling must encompass its entire geographic distribution, from eastern Canada to the southeastern United States and westward. Evidence of morphological plasticity across these diverse environments would indicate whether canalization is a fixed, species-level trait or a geographically variable outcome contingent upon localized evolutionary histories and environmental pressures.

Integration of Multidimensional Molecular Approaches

The most compelling future direction involves integrating geometric morphometrics with genomic tools to elucidate the mechanistic basis of the observed morphological variation. This integrative approach should prioritize several synergistic avenues:

Comparative Transcriptomics: Analyze differential gene expression during leaf development, targeting orthologs of key regulators like *RCO* and *JAGGED*, between populations from high- and low-altitude extremes.

Population Genomics: Utilize reduced-representation (e.g., ddRAD-seq) or whole-genome sequencing across the altitudinal cline to identify cryptic population structure, gene-flow barriers, or allele frequency clines associated with specific morphotypes.

Genetic Mapping: Conduct quantitative trait locus (QTL) mapping by crossing high- and low-complexity morphs to identify genomic regions governing leaf dissection.

Common Garden Experiments: Partition the genetic and environmental contributions to morphological diversity by growing progeny from across the range under standardized conditions. This is crucial for conclusively demonstrating phenotypic plasticity.

Application of Comparative Phylogenetic Frameworks

Finally, applying phylogenetic comparative methods (e.g., phylogenetic ANOVA) would allow for a robust assessment of whether the degree of phenotypic plasticity and developmental canalization are evolutionarily labile traits across the Apiaceae. If congeneric species pairs consistently exhibit disparities in morphological plasticity—as suggested by our *C. japonica–C. canadensis* comparison—it would imply that the capacity for canalization represents a fundamental axis of evolutionary divergence within herbaceous angiosperms.

Conclusions

This study provides the first data-driven, quantitative re-evaluation of the infraspecific taxonomy within the *Cryptotaenia japonica* complex. By applying an unsupervised, multivariate morphometric framework to a large dataset (n = 1,061), we demonstrate that the three traditionally recognized forms—f. *japonica*, f. *dissecta*, and f. *pinnatisecta*—do not constitute statistically distinct entities. Instead, they represent arbitrary segments along a continuous morphological cline primarily driven by altitudinal phenotypic plasticity.

Key findings supporting this conclusion include: (1) Principal Component Analysis revealed a gradient of leaf complexity without discrete clustering, with f. *dissecta* occupying an intermediate, bridging position; (2) Cluster validation metrics, notably a low Adjusted Rand Index (ARI = 0.088) and an optimal silhouette width at k= 2, are inconsistent with the traditional three-form classification; and (3) Generalized Additive Modelling identified a significant non-linear relationship between leaf complexity and elevation, with an inflection point near 850 m s.l.m, suggesting that leaf morphology varies along altitudinal gradients.

The stark contrast in leaf trait variation between the plastic East Asian *C. japonica* and the canalized North American *C. canadensis* further illustrates divergent evolutionary strategies following continental vicariance. Methodologically, this work underscores the power of unsupervised learning and geometric morphometrics in challenging *a priori* taxonomic hypotheses and revealing continuous patterns of variation often obscured by qualitative assessment.

In light of this evidence, we suggest a substantial taxonomic revision: *Cryptotaenia japonica* may be better recognized as a single, polymorphic species exhibiting a cline of leaf dissection in response to altitude. The forms f. *dissecta* and f. *pinnatisecta* should be treated as synonyms reflecting this altitudinal morphological variation. This reclassification moves the taxonomy of the complex from an artificial, discrete system towards a model that more accurately represents its continuous biological reality.

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