J Bio Res Biotech. August 2026. 24(2): 778-786

Content available at Bio-Research

Journal of Biological Research and Biotechnology
Journal Home page: https://bio-research.com.ng/index.php/home

ORIGINAL ARTICLE

Comparative anatomy of leaf, stem and petiole in *Lagenaria siceraria* (Cucurbitaceae) accessions from southwestern Nigeria: A Multivariate Approach

[1*]Yemisi O. Olagunju✉,[2]Jayeola A. Adeniyi, [3]Matthew O. Joshua, and [4]Olawuyi J. Odunayo.
[1] Biotechnology Unit, National Horticultural Research Institute, Ibadan, Oyo State, Nigeria.
[2] Plant Anatomy Unit, Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria.
[3] Fruits Program, National Horticultural Research Institute, Ibadan, Oyo State, Nigeria.
[4] Genetics and Molecular Biology Unit, Department of Botany, University of Ibadan, Ibadan, Oyo State, Nigeria.

ARTICLE INFO

Received: 17th June 2026.
Revised: 20th August 2026.
Accepted: 21st August 2026.
Published online: 31st August 2026
DOI: https://dx.doi.org/10.4314br.v24i2.36

e-ISSN: 2705-3822
print-ISSN: 1596-7409

Official. Faculty of Biological Sciences, University of Nigeria.

ABSTRACT

Intraspecific anatomical variation in domesticated plants is expected to concentrate in plastic, surface-exposed characters while core vascular and epidermal architecture remains stable, yet this prediction has rarely been tested across multiple organs simultaneously in a single accession collection. Transverse sections of the leaf midrib, stem and proximal and distal petioles were prepared from 45 accessions of *Lagenaria siceraria* sampled across four states of southwestern Nigeria and scored for 19 qualitative anatomical characters using a defined codebook. The study tested whether intraspecific anatomical variation in this domesticated cucurbit follows the pattern of peripheral variation against a conserved structural core, and whether anatomical groupings reflect geographic origin. Core architectural characters, including the bicollateral vascular bundle, uniseriate epidermis and striated cuticle, were invariant across all 45 accessions and all four organs, confirming a conserved structural core. Variation was concentrated in midrib outline, trichome complement, petiole outline and stem vascular arrangement. One accession, OY11, separated as a singleton cluster in three independent organ analyses, with a coherent cross-organ profile of reduced cortical elaboration that is unlikely to reflect sectioning artefact. Accessions did not cluster by state of geographic origin in any organ. The results confirm that intraspecific anatomical variation in *L. siceraria* follows the predicted pattern of peripheral variation against a conserved structural core, and identify trichome complement and petiole bundle arrangement as the most informative characters for distinguishing accessions within the species.

Keywords: comparative anatomy; intraspecific variation; *Lagenaria siceraria*; trichome complement; vegetative anatomy; vascular bundle arrangement

Cite this article: Olagunju O. Y., Jayeola, A. A., Matthew, O. J., Olawuyi, J. O. (2026). Comparative anatomy of leaf, stem and petiole in *Lagenaria siceraria* (Cucurbitaceae) accessions from southwestern Nigeria: a multivariate approach. *Journal of Biological Research and Biotechnology*, 24 (2), 778-786

✉**Corresponding author:** Olagunju Yemisi O. Email: olufolajiyemisi@yahoo.com. ORCID: 0009-0006-3645-0371. **DOI**: https://dx.doi.org/10.4314br.v24i2.36

1.0 INTRODUCTION

Lagenaria siceraria (Molina) Standl., the bottle gourd or calabash, is among the most ancient domesticated cucurbits, cultivated across tropical and subtropical regions for its edible immature fruits, hard-shelled mature fruits and oil-rich seeds (Mladenovic et al., 2017; Tas et al., 2019). Archaeological and genetic evidence places the centre of domestication in Africa, where the species has formed part of the smallholder food and utensil economy for at least three millennia (Kistler et al., 2014). In southwestern Nigeria, *L. siceraria* is maintained as a backyard and intercrop species across Ondo, Ogun, Osun and Oyo states, where farmers distinguish landraces principally by fruit shape, seed colour and culinary use. The morphological diversity of these landraces at the whole-plant level is well documented, yet their internal anatomical structure has never been formally characterised at the intraspecific level.

Vegetative anatomy is an established and objective tool for characterising plant diversity, identifying taxonomically diagnostic features and documenting structural variation that is not visible in external morphology (Ekeke et al., 2017). Within Nigerian plant taxa specifically, leaf epidermal and petiole characters including trichome types, petiole outline and vascular bundle organisation have demonstrated taxonomic utility at both the species and genus level (Okanume et al., 2022), and microstructural features such as trichome complement have proven reliable for plant identification and authentication (Abdullahi et al., 2021). In the Cucurbitaceae, characters including leaf midrib outline, stem vascular arrangement, petiole bundle architecture and trichome complement have been shown to vary informatively among genera and species, providing characters of systematic and ecological significance (Filartiga et al., 2022; Baderinwa-Adejumo & Adenegan-Alakinde, 2023). Previous anatomical studies of *L. siceraria* and related cucurbits have focused on interspecific or intergeneric comparisons for taxonomic purposes; none has examined anatomical variation systematically within a single species across a large collection of accessions. This is a critical gap, because intraspecific anatomical variation captures a dimension of diversity that agro-morphological and molecular marker studies cannot address directly.

Recent multivariate studies of bottle gourd germplasm have documented substantial intraspecific diversity in fruit shape, seed morphology and molecular markers at regional and global scales (Flores-Chacon et al., 2024; Kumar et al., 2024; Mahapatra et al., 2022), but these investigations examined only external and molecular features. Whether internal anatomical characters - the structural organisation of the leaf midrib, stem cortex and petiole vasculature - vary meaningfully among accessions, and whether that variation is geographically structured, has not been tested. This question is not trivial: if anatomical characters discriminate among accessions independently of fruit and seed morphology, they provide a complementary and reproducible basis for landrace characterisation that does not depend on fruit development stage or post-harvest condition.

Theory predicts that within-species morphological variation concentrates in plastic, surface-exposed features such as trichome complement, outline shape and palisade organisation, while core architectural characters such as vascular bundle type, cuticle pattern and epidermis layering remain stable across accessions (Puglielli et al., 2024; Westerband et al., 2021). Testing this prediction in *L. siceraria* requires a multivariate analytical framework capable of separating informative from invariant characters across mixed nominal and ordinal data - a framework that has been applied to bottle gourd external morphology but never to its internal anatomy. The present study addresses this gap directly. Its three objectives were: (1) to document the qualitative anatomy of the leaf midrib, stem, proximal petiole and distal petiole in 45 *L. siceraria* accessions from four southwestern Nigerian states; (2) to identify which anatomical characters are invariant and which discriminate among accessions, using a codebook applied uniformly across organs; and (3) to test whether variable characters group accessions into coherent anatomical clusters and whether cluster membership corresponds to state of geographic origin, using Gower-distance hierarchical clustering and principal component analysis applied independently to each organ.

2.0 MATERIALS AND METHODS

2.1 Plant material and sampling

Accessions of *Lagenaria siceraria* (Molina) Standl. were assembled from four states of southwestern Nigeria: Ogun (OG, n = 11), Oyo (OY, n = 12), Osun (OS, n = 12) and Ondo (OD, n = 10), through farmer-to-farmer collections and local market surveys conducted between January and June 2023. Voucher specimens were deposited at the herbarium of the Department of Botany, University of Ibadan, Nigeria. Mature plants from each accession were raised under uniform conditions at the NIHORT research field, and tissues were sampled from at least three plants per accession. Stem internodes were collected at the third node below the apex; fully expanded leaves and their petioles were collected from the same node, with the petiole divided into proximal (within 2 cm of the stem) and distal (within 2 cm of the leaf blade) segments. Per-organ sample sizes used in the multivariate analyses are reported in Table 2.

2.2 Tissue fixation, sectioning and staining

Fresh tissues were trimmed to 5 – 10 mm segments and fixed in formalin-acetic acid-alcohol (FAA; 10:5:50, with 35 mL distilled water per 100 mL fixative) for 48 h. Dehydration proceeded through a graded ethanol series (50, 70, 90, 95 and 100%, 1 h each), followed by clearing in xylene and embedding in paraffin wax (Che et al., 2018). Transverse sections of 20-30 µm were cut with a Reichert sledge microtome (Reichert, Vienna, Austria), de-waxed in xylene, rehydrated, stained with 1% aqueous safranin O (3 min) to highlight lignified walls, counterstained with 0.05% toluidine blue (3 min) for general tissue contrast, and mounted in 25% glycerol (Ojo et al., 2023).

2.3 Microscopy and image capture

Sections were examined under an Accu-Scope trinocular light microscope (Accu-Scope Inc., Commack, NY, USA) at 40 x and 100 x total magnification, and photomicrographs were captured with an AmScope MA1000 CMOS camera (3.2 MP; AmScope, Irvine, CA, USA). For each accession-organ combination, two to five sections were examined; the most representative section was selected for character scoring, and a corresponding micrograph was retained for illustration.

2.4 Anatomical character scoring

Nineteen anatomical characters were scored across the four organ types: eight for the leaf midrib (cuticle pattern, epidermis layers, epidermis cell shape, trichome complement, palisade packing, midrib outline, maximum collenchyma layers, vascular-bundle arrangement), seven for the stem (outline, cuticle pattern, epidermis layers, trichome complement, maximum collenchyma layers, maximum sclerenchyma layers, vascular-bundle arrangement), and seven for each petiole region (outline, surface convexity, cuticle pattern, epidermis cell shape, trichome complement, maximum collenchyma layers, vascular-bundle arrangement). Characters were defined *a priori* from the descriptive vocabulary in published cucurbit anatomy (Filartiga et al., 2022; Baderinwa-Adejumo & Adenegan-Alakinde, 2023) and coded as nominal integers, except for layer-count characters treated as ordinal. The full codebook is given in Table 1. Sections where one or more characters could not be scored due to sectioning damage were excluded from that organ's analysis.

2.5 Multivariate analysis

Analyses were run independently per organ to avoid confounding organ identity with accession identity and to respect unequal sample coverage across organs. Characters invariant across all accessions of a given organ were excluded before analysis, as they contribute zero variance. Pairwise dissimilarities were computed using Gower's coefficient (Gower, 1971), the standard distance measure for mixed nominal and ordinal data, using the daisy function in the cluster package (Maechler et al., 2026) in R v4.3 (R Core Team, 2023), with type set to 'nominal' for qualitative characters and 'ordratio' for layer counts. Hierarchical clustering used the unweighted pair-group method with arithmetic mean (UPGMA, hclust with method = 'average'), with dendrograms cut at k = 3 consistently across all organs. Principal component analysis was performed on standardised character matrices using FactoMineR (Le et al., 2008). The R script is provided as Supplementary S1; per-organ character matrices are in Supplementary S2.

3.0 RESULTS

3.1 Descriptive anatomy

All four organs showed a high degree of structural conservation, with most characters either invariant or expressed in few states across the collection. Per-accession observations are presented in Table S1 (leaf), Table S2 (stem), Table S3 (proximal petiole) and Table S4 (distal petiole).. In every accession, the cuticle was thin, undulating and striated, the epidermis was uniseriate (except localised multiseriate patches in OD01, OD05, OS04, OS05, OY11 and OY12 in the distal petiole), and the vascular bundles were of the open, conjoint, bicollateral type. These invariant characters were excluded from the multivariate analyses.

Table **1.** Codebook of anatomical characters and their states used to score 45 *Lagenaria siceraria* accessions from southwestern Nigeria. Characters scored on the leaf, stem and petiole (both proximal and distal regions). The integer code used in the data matrix is given in parentheses after each state.

Organ	Character	States and codes	Type
Leaf	Cuticle pattern	Thin, undulating, striated (1); variable undulation, striated (2)	nominal
Leaf	Epidermis layers	Uniseriate (1)	invariant
Leaf	Epidermis cell shape	Mixed shapes (1)	invariant
Leaf	Trichome complement	SM + SG (1); SM + GH (2); SM + MGH + SG (3)	nominal
Leaf	Palisade packing	1-2 layers, loose (1); 2-3 layers (2); tightly packed (3); highly packed (4)	ordinal
Leaf	Midrib outline	Jug, both convex (1); jug, mixed (2); near-flat/both concave (3); not specified (0)	nominal
Leaf	Collenchyma layers (max)	Layer count (continuous)	ordinal
Leaf	Vascular-bundle arrangement	Vertical, 2-3 cortical (1); radial (2)	nominal
Stem	Outline	Angular (1); angular, less pronounced (2)	nominal
Stem	Cuticle pattern	Thin, undulating, striated (1)	invariant
Stem	Epidermis layers	Uniseriate (1)	invariant
Stem	Trichome complement	SM only (1); SM + MC (2); SM + G (3)	nominal

Organ	Character	States and codes	Type
Stem	Collenchyma layers (max)	Layer count (continuous)	ordinal
Stem	Sclerenchyma layers (max)	Layer count (continuous; 0 = not specified)	ordinal
Stem	Vascular-bundle arrangement	Scattered (1); scattered approaching radial (2); radial (3)	nominal
Petiole	Outline	Apple-shaped (1); circular (2); circular-oval/oval (3); pear (4); apple-arc (5); circular-apple (6); arc/arc-circular (7); apple-circular (8)	nominal
Petiole	Surface convexity	Both convex (1); abaxial convex, adaxial concave (2); adaxial flat, abaxial convex (3); both concave (4)	nominal
Petiole	Cuticle pattern	Thin, undulating, striated (1)	invariant
Petiole	Epidermis cell shape	Mixed shapes (1); mixed with polygonal (2); mixed with elongated (3)	nominal
Petiole	Trichome complement	SM + SG (1); SM + G (2); S + MC + G (3)	nominal
Petiole	Collenchyma layers (max)	Layer count (continuous)	ordinal
Petiole	Vascular-bundle arrangement	Radial simple (1); radial + ridge (2); radial + 2 ridge (3); radial + internal + ridge (4); radial + 2 arms (5); radial + ridge + internal (6); scattered (7); more-or-less radial (8)	nominal

SM = simple multicellular trichome; SG = short glandular trichome; GH = glandular hair; MGH = multicellular glandular hair; MC = multicellular; G = glandular; S = single trichome.

Leaf transverse sections (Figure 1) showed two cuticle patterns: thin-undulating-striated in 23 of 24 accessions and variable-undulation-striated in OY06 alone. Three trichome complements were recorded: simple-multicellular plus short-glandular (SM + SG) in 20 accessions, simple-multicellular plus glandular-hair (SM + GH) in OY11 only, and the more elaborate SM + MGH + SG in OD01, OD03, OD04 and OY03. The midrib outline was jug-shaped with both surfaces convex in 20 of 24 accessions; OY11 showed a near-flat to both-concave outline, while OY03, OY04 and OD03 fell within the jug-shaped range but had denser palisade and a more elaborate trichome complement. Palisade tissue was loosely packed (1-2 layers) in 19 accessions and denser in five (OS01, OS08, OY04, OD03, OD04). Maximum collenchyma counts ranged from three to ten layers, with OY03 recording the highest count. Stem sections (Figure 2) were uniformly angular in outline except OY11, which was angular but less pronounced. Trichome complements were divided into three states: SM-only in OY11, SM + MC in OY12, and SM + G in the remaining 20 accessions. Collenchyma ranged from three layers (OY12) to eight (OG05, OG06, OS01, OS06), and sclerenchyma, where reliably scored, from four to ten layers. Vascular bundles were scattered in 18 of 22 accessions, scattered-approaching-radial in OS05, and radial in OY10, OY11, OY12 and OS10. Petiole sections (Figures 3 and 4) showed the greatest outline diversity. In the proximal region, eight accessions had a circular or circular-oval outline with both surfaces convex (OS08, OD08, OG08, OS04, OY07, OY12, OY10, OG12), six had an apple-shaped outline with abaxial-convex/adaxial-concave surfaces (OY06, OY09, OS05, OS03, OD03, OY02), and OY11 had a pear-shaped outline with both surfaces concave. In the distal region, the apple-shaped outline with abaxial-convex/adaxial-concave surface dominated (15 of 20 accessions); five others diverged in outline (arc, arc-circular, apple-arc, circular-apple, circular-oval) or surface convexity (both convex). Distal-petiole vascular organisation was the most complex of all organs, with eight arrangements recorded ranging from simple radial to compound patterns with ridge bundles, internal bundles or arm bundles superimposed on the radial core. Sclerenchyma was present only in the proximal petiole of OY06 (at least 5 layers).

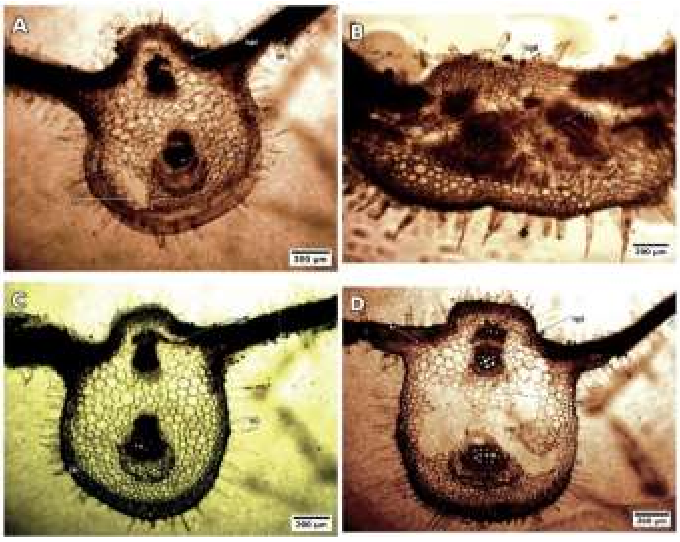

*Figure **1.** Leaf midrib - Transverse sections from four accessions showing the range of midrib forms. OY02 is the typical jug-shaped, both-convex type (cluster 1); OY11 is the near-flat/both-concave outlier (cluster 2); OD03 has an elaborate trichome complement and denser palisade (cluster 3); OS08 is a dense-palisade cluster-1 variant. 40× magnification, scale bars 200 µm.*

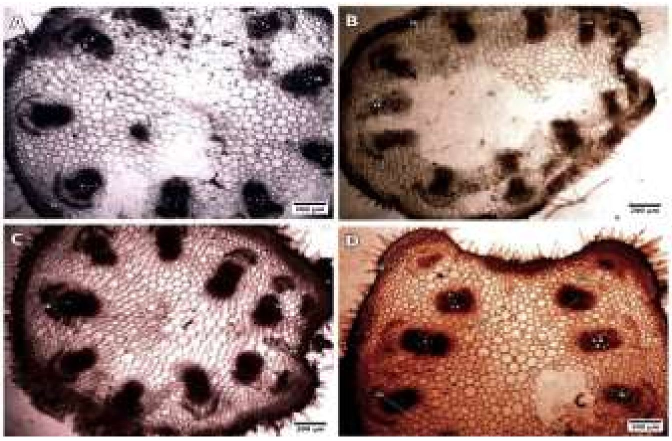

*Figure **2.** Stem - Sections showing vascular arrangement and trichome differences. OG02 is the typical scattered-bundle type (cluster 3); OY11 shows the atypical radial arrangement and less-pronounced outline (cluster 2); OY12 is a single-bundle close-up (cluster 1); OS01 is another typical scattered type. Scale bars 200 µm (100 µm in the close-up)*

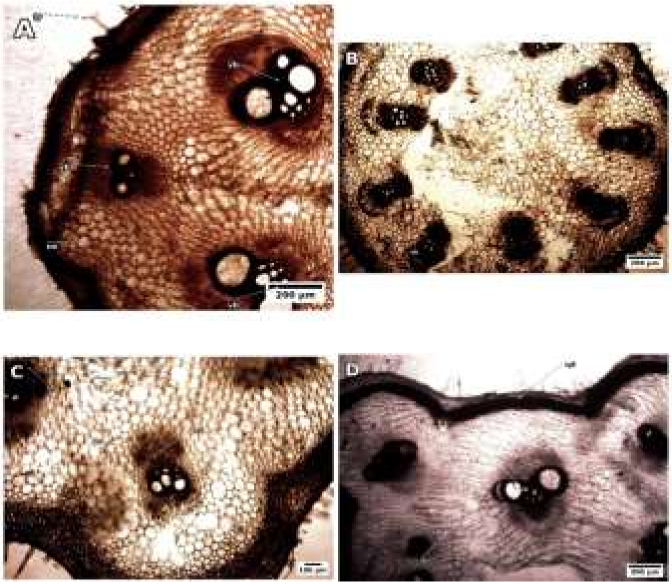

Figure **3.** *Proximal petiole - Sections illustrating outline diversity. OG08 is circular with a peripheral bundle ring (cluster 1); OY11 is the elongated asymmetric outlier (cluster 2); OD03 is apple-shaped (cluster 3); OS05 is an apple-shaped variant with distinct ridge bundles. Scale bars 200 µm.*

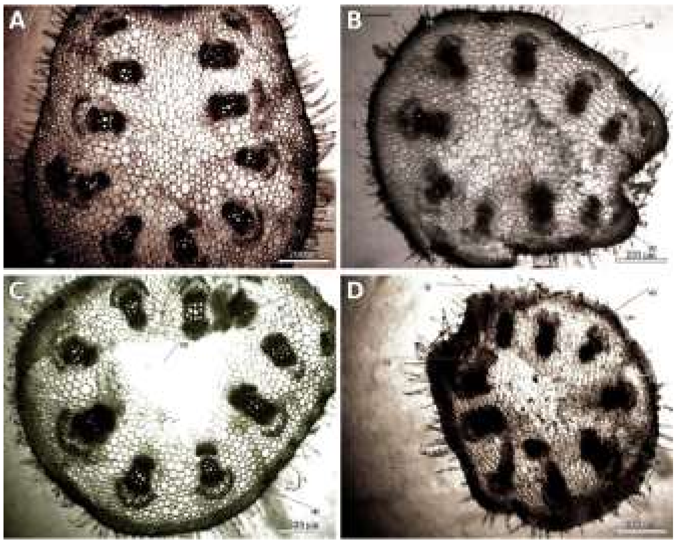

Figure **4.** *Distal petiole* **-** *Sections showing the four outline states. OY02 is the dominant apple-shaped type (cluster 2); OY11 is pear-shaped (outlier); OS12 is circular-oval with both surfaces convex (cluster 1); OD05 is circular-apple (cluster 3). Scale bars 200 µm.*

3.2 Multivariate analysis of variance structure

Per-organ PCA results are summarised in Table 2. The stem analysis was dominated by a single axis (PC1 = 62.4%), with PC1 and PC2 together accounting for 81.7% of variance. In the leaf and distal-petiole analyses, variance was more evenly distributed (PC1 = 33.2% in both; PC1 + PC2 = 59.5% and 54.8% respectively), and the proximal petiole was the most balanced (PC1 = 27.0%; PC1 + PC2 = 50.5%). Scree plots and PC1 loading bar charts for each organ are shown in Supplementary Figure S1 and S2, respectively.

Table **2.** Summary of per-organ multivariate analyses. PC1 and PC2 are the percentages of total variance explained by the first and second principal components. Informative characters are those retained after invariant characters were excluded.

Organ	**n**	**Informative characters**	**PC1 (%)**	**PC2 (%)**	**PC1 + PC2 (%)**	**Cluster sizes (k = 3)**
Leaf	24	6	33.2	26.3	59.5	20 / 1 / 3
Stem	22	5	62.4	19.3	81.7	20 / 1 / 1
Proximal petiole	15	6	27.0	23.5	50.5	8 / 1 / 6
Distal petiole	20	6	33.2	21.5	54.8	18 / 1 / 1

For the leaf, midrib outline (loading = 0.92), trichome complement (0.70) and vascular-bundle arrangement (0.62) drove PC1, with cuticle pattern contributing weakly in the opposite direction (-0.21). The stem PC1 contrasted trichome complement (0.93) and sclerenchyma layer count (0.75) against stem outline (-0.81) and vascular-bundle arrangement (-0.79), placing accessions with SM + G trichomes, more sclerenchyma, angular outline and scattered bundles at one end and OY11 and OY12 at the other. For the proximal petiole, PC1 contrasted collenchyma maximum and epidermis cell shape (positive loadings) against surface convexity and outline (negative loadings). For the distal petiole, PC1 contrasted surface convexity (0.90) against trichome complement (-0.84).

3.3 Multivariate analysis of clustering

PCA biplots of all four organ datasets showed broad overlap of accessions across state groups along both principal axes, with character vectors indicating the main directions of anatomical variation within each organ (Supplementary Figure S3). The stem biplot showed the greatest separation along Dim 1, consistent with its higher PC1 variance, while the petiole and leaf biplots showed more diffuse scatter with no clear geographic clustering. Cutting the four UPGMA dendrograms at k = 3 (Figure 5) produced structures dominated by one large core cluster, with one or a few accessions separating as small outlier clusters. In the leaf (Figure 5A), 20 of 24 accessions formed a core cluster with the jug-shaped convex midrib, SM + SG trichome complement and 1-2-layer palisade. OY11 formed a singleton cluster based on its near-flat/both-concave midrib and SM + GH trichomes. OY03, OY04 and OD03 formed a third cluster based on their elaborate SM + MGH + SG trichome complement combined with denser palisade. In the stem (Figure 5B), 20 of 22 accessions clustered together; OY11 and OY12 each separated as singletons based on atypical trichome complements and radial bundle arrangement.

The two petiole analyses produced more balanced groupings. The proximal-petiole dendrogram (Figure 5C) resolved a circular/circular-oval cluster with both-convex surfaces (n = 8: OS08, OD08, OG08, OS04, OY07, OY12, OY10, OG12), a singleton (OY11, pear-shaped, both surfaces concave), and an apple-shaped cluster with abaxial-convex/adaxial-concave surfaces (n = 6: OY06, OY09, OS05, OS03, OD03, OY02). The distal-petiole dendrogram (Figure 5D) was dominated by an 18-accession core cluster with the apple-shaped outline and abaxial-convex/adaxial-concave surface state; OS12 and OD05 separated as singletons based on unusual trichome complement and circular-apple outline, respectively.

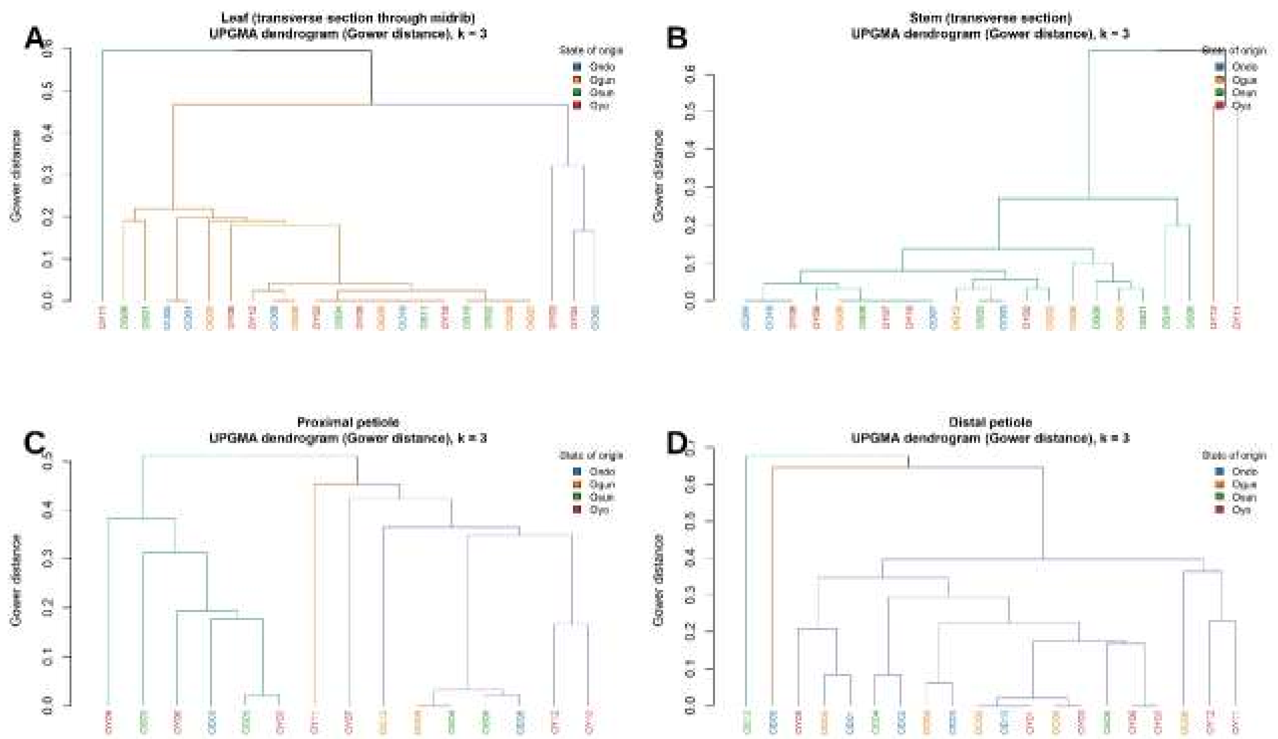

*Figure **5**. UPGMA dendrograms of Lagenaria siceraria accessions based on Gower distance, cut at k = 3. Tip labels are coloured by state of origin: Ondo (blue), Ogun (orange), Osun (green), Oyo (red); branches are coloured by cluster membership. (A) Leaf (24 accessions, 6 informative characters). (B) Stem (22 accessions, 5 characters). (C) Proximal petiole (15 accessions, 6 characters). (D) Distal petiole (20 accessions, 6 characters).*

3.4 Reproducibility of outliers across organs

OY11 was an outlier in three independent organ analyses (leaf, stem and proximal petiole) and occupied a distinctive position in the distal-petiole analysis. Across organs, OY11 was characterised by a near-flat to both-concave midrib, SM + GH trichome complement, angular-but-less-pronounced stem outline, and pear-shaped/both-concave proximal petiole. OD03 and OY02 co-occurred in the third cluster of both the leaf and proximal-petiole analyses, forming a secondary cross-organ pattern. In all four organ analyses, accessions from Ondo, Ogun, Osun and Oyo states were distributed across all clusters (Figure 5), and no cluster was dominated by accessions from a single state.

4.0 DISCUSSION

This study provides the first systematic, multi-organ anatomical characterisation of *L. siceraria* accessions at the intraspecific level, and its findings make three contributions that have not previously been reported for this species or for any other cucurbit at comparable resolution. First, intraspecific anatomical variation in *L. siceraria* follows the predicted pattern of peripheral variation against a conserved structural core, confirming at the organ level a principle previously demonstrated only from whole-plant external traits in this species. Second, one accession, OY11, diverges reproducibly across three independently analysed organs with a coherent developmental profile, providing the first anatomically grounded evidence of a distinctly divergent landrace in the Nigerian bottle gourd collection. Third, anatomical cluster membership shows no correspondence with state of geographic origin in any organ, extending the geographic non-structure previously documented from morphological and molecular markers to internal anatomical organisation.

The open, conjoint, bicollateral vascular bundle remained invariant across all 45 accessions and all four organs. Previous anatomical work on *L. siceraria* and related cucurbits has reported this character at the family and genus level, but its stability within a single species across a large accession collection has not previously been demonstrated quantitatively. Ajuru and Okoli (2013) observed comparable stability in vascular-bundle type and epidermis layering when comparing several cucurbit genera in Nigeria, but their comparison was intergeneric rather than intraspecific. Baderinwa-Adejumo and Adenegan-Alakinde (2023), working on five *Cucurbita* species in southwestern Nigeria, found constancy in epidermal layering across species while identifying midrib shape as the most taxonomically informative axis of variation, a finding mirrored here at the intraspecific level, where midrib outline loaded most strongly on PC1 (0.92). The present data therefore confirm that the architectural hierarchy observed at the interspecific level, stable vascular and epidermal core with variable surface features, also holds within *L. siceraria* at the accession level.

Bicollateral vascular bundles, uniseriate epidermis and striated cuticle are primary developmental products established early in organogenesis that do not remodel in response to environmental change. Westerband et al. (2021) demonstrated in a broad cross-taxon survey that within-species variation accumulates in plastic, late-differentiated surface features rather than in structural cores. The present data confirm this prediction specifically for a domesticated cucurbit: of the 29 organ-character combinations scored, 12 (41%) were entirely invariant, a proportion that reflects genuine developmental constraint rather than insufficient sampling. This finding is significant because it establishes, for the first time in *L. siceraria*, a clear boundary between anatomically fixed and variable characters, with practical implications for future anatomical characterisation of additional accessions.

Trichome complement emerged as the single most diagnostically informative character across organs, loading most strongly on PC1 in both the leaf (0.70) and stem (0.93) analyses. This finding is novel for *L. siceraria*: previous anatomical descriptions of the species noted the presence of trichomes but did not systematically differentiate complement types among accessions or quantify their discriminating power. Three leaf states were recorded: SM + SG in 20 of 24 accessions, SM + GH restricted to OY11, and SM + MGH + SG in four accessions. In the stem, 20 accessions bore SM + G, OY12 bore SM + MC, and OY11 bore SM alone. Patel et al. (2021) found drastic trichome differences between lentil genotypes from identical geographic origins, consistent with the absence of state-of-origin structure recorded here. Watts and Kariyat (2021) showed across 14 *Solanum* species that subtype-level trichome distinctions carry discriminating power that the simple glandular versus non-glandular classification misses; the contrast between SM + GH in OY11 and SM + SG in typical accessions demonstrates this subtype-level discrimination operating within a single *L. siceraria* collection. The association between the SM + GH complement and OY11's other divergent characters across organs suggests a coherent developmental syndrome whose genetic and functional basis warrants direct investigation.

The four organs differed in how many independent axes of variation their character sets expressed, a result that has not previously been reported for any cucurbit at the intraspecific level. The stem required only one dominant axis (PC1 = 62.4%; PC1 + PC2 = 81.7%), while leaf and petiole analyses required three to four axes to account for 75% of variance. This contrast reflects fundamental differences in the functional demands placed on these organs. Rowe and Speck (2015) noted that climbing-vine stems must balance tensile stiffness against torsional flexibility, which restricts viable structural configurations to a narrow range. The essentially invariant angular outline of *L. siceraria* stems, confirmed across South African landraces by Nkosi et al. (2022), is consistent with this mechanical constraint, and the four characters that did vary, trichome complement, sclerenchyma, bundle arrangement and stem outline in OY11, are precisely those most susceptible to developmental or environmental modulation rather than fixed structural change. The petiole, by contrast, must integrate mechanical support, hydraulic conductance, surface defence and lamina positioning simultaneously. Filartiga et al. (2022) showed that petiole anatomy responds independently to leaf area and plant height across 95 European woody species; the multi-dimensional petiole variation observed here within a single species is consistent with this functional complexity operating at the intraspecific scale and represents the first documentation of this contrast between stem and petiole anatomical dimensionality in *L. siceraria*.

OY11 separated as a singleton cluster in three independent organ analyses. Its distinguishing profile, near-flat/both-concave midrib, SM-only trichomes, radial rather than scattered stem bundle arrangement, and asymmetric elongated petiole outline, is internally coherent across organs and points to an accession with reduced lateral expansion of cortical and subepidermal tissues. The reproducibility of this divergence across three independently analysed organs rules out sectioning artefact: a preparation problem would affect one organ's data, not all three. No previous study of *L. siceraria* has identified a consistently divergent accession across multiple anatomical organs simultaneously, making OY11 a novel finding of this study. Awala and Obiri (2023), applying taximetric analysis to 24 Nigerian *L. siceraria* landraces, concluded that the species should be maintained as a single taxon with dissimilarity confined to fruit and seed shape. Mahapatra et al. (2022) and Flores-Chacon et al. (2024) found individual morphological outliers in large international collections without evidence for subspecific separation. OY11 fits this pattern of intraspecific divergence without taxonomic separation, representing a landrace whose anatomical distinctiveness warrants targeted molecular and morphological follow-up.

The distal petiole showed eight distinguishable vascular bundle arrangements, more than any other organ in this study and more than previously documented within any single cucurbit species. Ekeke et al. (2015) found petiole bundle number and arrangement to vary more informatively among cucurbit genera than epidermis or cortex characters, and the present data extend this finding from the intergeneric to the intraspecific level. Filartiga et al. (2022) demonstrated that petiole vascular bundle area scales with leaf area across woody species because of the hydraulic demand imposed by a larger lamina; variation in lamina area among *L. siceraria* accessions could produce variation in bundle elaboration independent of genetic divergence, a hypothesis testable by quantitative measurement of bundle area and cortex thickness. The co-occurrence of elaborate bundle arrangements with the apple-shaped outline and abaxial-convex/adaxial-concave surface state suggests that petiole bundle organisation and outline geometry are not anatomically independent in this species.

Accessions from all four states mixed across clusters in every organ analysis. Mahapatra et al. (2022) found the same result across 91 Indian genotypes from 14 agro-climatic zones, and Flores-Chacon et al. (2024) across 19 accessions from nine countries. Buthelezi et al. (2023) and Nkosi et al. (2022) observed geographic non-structure in South African landraces from fruit and agromorphological traits. The present study is the first to demonstrate geographic non-structure in *L. siceraria* from internal anatomical data, adding a new dimension to the established pattern and confirming that free farmer seed exchange erodes geographic structure even in characters outside the scope of direct selection.

Per-organ sample sizes ranged from 15 to 24 accessions, and only seven accessions had full four-organ coverage. The cross-organ signal for OY11 is strong because it holds across three independent analyses, but less extreme divergences cannot be assessed at equivalent confidence. Qualitative scoring from single sections per accession, while standard in descriptive anatomy, cannot resolve quantitative differences in cell dimensions. Extending to cell-level measurements using image analysis, increasing accession coverage across additional agro-ecological zones of Nigeria, and integrating anatomical data with molecular markers for the same accessions would substantially strengthen future inferences.

Conclusion

This study provides the first multivariate anatomical characterisation of *L. siceraria* accessions at the intraspecific level and the first to examine internal anatomy simultaneously across four vegetative organs in a large Nigerian collection. Three findings stand out. The species is anatomically conservative, with 41% of organ-character combinations entirely invariant and core architectural characters including the bicollateral vascular bundle, uniseriate epidermis and striated cuticle stable across all 45 accessions, confirming the conserved structural core predicted by intraspecific trait variation theory. Trichome complement and petiole bundle arrangement are the most informative characters for distinguishing accessions, a result not previously established for this species. Accession OY11 is a reproducible anatomical outlier across three independent organ analyses with a coherent cross-organ developmental profile, making it the first anatomically characterised divergent landrace in the Nigerian *L. siceraria* collection and a priority candidate for molecular and morphological follow-up. Anatomical cluster membership showed no correspondence with state of geographic origin in any organ, extending the geographic non-structure previously documented from external morphology and molecular markers to internal anatomical organisation for the first time in this species. The codebook, character matrices and analysis code provided as supplementary material establish a reproducible framework for future intraspecific anatomical studies of *L. siceraria* and other Nigerian cucurbits.

Acknowledgements

The authors acknowledge the support of the Biotechnology Unit, National Horticultural Research Institute (NIHORT), Nigeria, and the Department of Botany, University of Ibadan, for laboratory facilities and technical assistance. The authors also thank the smallholder farmers of Ogun, Oyo, Osun and Ondo states for providing accession material.

Conflict of interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The study was supported by the authors' personal resources and institutional laboratory facilities at the Department of Botany, University of Ibadan, and the National Horticultural Research Institute (NIHORT), Nigeria.

Data availability

The data supporting the findings of this study are available from the corresponding author (Olagunju Y.O., olufolajiyemisi@yahoo.com) upon reasonable request. The R analysis script is provided as Supplementary S1 and the per-organ anatomical character matrices as Supplementary S2.

Author contributions

Conceptualisation: OYO and OOJ Methodology. OYO and JAA Investigation. OYO and MJO Data curation. OYO Formal analysis. OYO and JAA Supervision. OOJ and JAA Writing - original draft. OYO Writing – review and editing: all authors. All authors have read and agreed to the submitted version of the manuscript.

REFERENCES

Abdullahi, A. A., Abdullah, A. S. A., Aluko, T. A., Adebunmi, A. S., & Abdulquadri, S. (2021). The use of microstructures in the authentication of powdered drug plants. *Journal of Biological Research and Biotechnology*, *19*(2), 1297–1305. https://doi.org/10.4314/br.v19i2.3

Ajuru, M. G., & Okoli, B. E. (2013). The morphological characterisation of the melon species in the family Cucurbitaceae Juss., and their utilisation in Nigeria. *International Journal of Modern Botany*, *3*(2), 15–19. https://doi.org/10.5923/j.ijmb.20130302.01

Awala, F. O. N., & Obiri, E. E. (2023). Taximetrics: A tool in the morphological characterisation of *Lagenaria siceraria* (Mol.) Standl. *FNAS Journal of Scientific Innovations*, *5*(2), 78–90.

Baderinwa-Adejumo, A. O., & Adenegan-Alakinde, T. A. (2023). Vegetative anatomy of five domesticated members of the genus *Cucurbita* (Linn.) in Southwestern Nigeria. *Research in Plant Biology*, *13*, 29–36.

https://doi.org/10.25081/ripb.2023.v13.8575

Buthelezi, L. G., Mavengahama, S., Sibiya, J., & Ntuli, N. R. (2023). Variation in shoot, peduncle and fruit growth of *Lagenaria siceraria* landraces. *Plants*, *12*(3), 532. https://doi.org/10.3390/plants12030532

Che Amri, C. N. A., Tajudin, N. S., Shahari, R., Azmi, F. M., Talip, N., & Mohamad, A. L. (2018). Comparative leaf anatomy of selected medicinal plants in Acanthaceae. *International Islamic University Malaysia Medical Journal Malaysia*, *17*(2). https://doi.org/10.31436/imjm.v17i2.944

Ekeke, C., Agbagwa, I. O., & Ogazie, A. C. (2017). Comparative anatomy of stem, petiole and flower stalks and its significance in the taxonomy of some members of Cucurbits. *Jordan Journal of Biological Sciences*, *10*(3), 185–191. https://doi.org/10.5897/AJPS11.309

Ekeke, C., Agogbua, J., & Okoli, B. E. (2015). Comparative anatomy of tendril and fruit stalk in Cucurbitaceae Juss. from Nigeria. *International Journal of Biological and Chemical Sciences*, *9*(4), 1875–1887. https://doi.org/10.4314/ijbcs.v9i4.13

Filartiga, A. L., Klimes, A., Altman, J., Nobis, M. P., Crivellaro, A., Schweingruber, F., & Dolezal, J. (2022). Comparative anatomy of leaf petioles in temperate trees and shrubs: The role of plant size, environment and phylogeny. *Annals of Botany*, *129*(5), 567–582. https://doi.org/10.1093/aob/mcac014

Flores-Chacon, S., Carreno, G., Maldonado, C., & Contreras-Soto, R. (2024). Comprehensive assessment of morphological diversity in bottle gourd (*Lagenaria siceraria*) accessions: A focus on roots and morpho-agronomic traits. *Diversity*, *16*(3), 136. https://doi.org/10.3390/d16030136

Gower, J. C. (1971). A general coefficient of similarity and some of its properties. *Biometrics*, *27*, 857–871. https://doi.org/10.2307/2528823

Kistler, L., Montenegro, A., Smith, B. D., Gifford, J. A., Green, R. E., Newsom, L. A., & Shapiro, B. (2014). Transoceanic drift and the domestication of African bottle gourds in the Americas. *Proceedings of the National Academy of Sciences of the United States of America*, *111*, 2937–2941. https://doi.org/10.1073/pnas.1318678111

Kumar, R., Kumar, R., Prasad, B. D., Kumar, J., & Bamaniya, B. S. (2024). Genetic diversity of bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] genotypes of Eastern India through agro-morphological traits and ISSR markers: Implication for future breeding. *Genetic Resources and Crop Evolution*, *71*(2), 873–891. https://doi.org/10.1007/s10722-023-01673-5

Le, S., Josse, J., & Husson, F. (2008). FactoMineR: An R package for multivariate analysis. *Journal of Statistical Software*, *25*, 1–18. https://doi.org/10.18637/jss.v025.i01

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., & Hornik, K. (2026). *cluster: Cluster Analysis Basics and Extensions* (R package version 2.1.8.2) [Software]. https://CRAN.R-project.org/package=cluster. https://doi.org/10.32614/CRAN.package.cluster

Mahapatra, S., Sureja, A. K., Behera, T. K., & Verma, M. (2022). Assessment of genetic diversity of ninety-one bottle gourd [*Lagenaria siceraria* (Mol.) Standl.] genotypes from fourteen different agro-climatic zones of India using agro-morphological traits and SSR markers. *Molecular Biology Reports*, *49*(7), 6367–6383. https://doi.org/10.1007/s11033-022-07389-4

Mladenovic, E., Berenji, J., Ognjanov, V., Ljubojevic, M., & Cukanovic, J. (2017). Genetic variability of bottle gourd *Lagenaria siceraria* (Mol.) Standley and its morphological characterisation by multivariate analysis. *Archives of Biological Sciences*, *69*, 481–490. https://doi.org/10.2298/ABS160608055M

Nkosi, L. S., Ntuli, N. R., & Mavengahama, S. (2022). Morpho-agronomic evaluation of *Lagenaria siceraria* landraces and their F1 populations. *Plants*, *11*(12), 1558. https://doi.org/10.3390/plants11121558

Ojo, F. M., Adelalu, K. F., Ariyo, A. C., & Omoyeni, B. O. (2023). Comparative anatomical studies of four accessions of *Bambusa vulgaris* Schrad. ex J.C. Wendl. collected in Ile-Ife, Osun State, Nigeria. *Science Letters*, *11*(1), 25–34. https://doi.org/10.47262/SL/11.1.132023200

Okanume, O. E., Gabriel, A. S., & Adekunle, O. O. (2022). Leaf epidermal and petiole anatomical features as taxonomic characters in some *Vernonia* species in Nigeria. *Journal of Biological Research and Biotechnology*, *20*(1), 1388–1397. https://doi.org/10.4314/br.v20i1.1

Patel, I., Gorim, L. Y., Tanino, K., & Vandenberg, A. (2021). Diversity in surface microstructures of trichomes, epidermal cells, and stomata in lentil germplasm. *Frontiers in Plant Science*, *12*, 697692. https://doi.org/10.3389/fpls.2021.697692

Puglielli, G., Bricca, A., Chelli, S., Petruzzellis, F., Acosta, A. T. R., Bacaro, G., et al. (2024). Intraspecific variability of leaf form and function across habitat types. *Ecology Letters*, *27*(3), e14396. https://doi.org/10.1111/ele.14396

R Core Team. (2023). *R: A language and environment for statistical computing* [Software]. R Foundation for Statistical Computing. https://www.R-project.org/

Rowe, N., & Speck, T. (2015). Stem biomechanics, strength of attachment, and developmental plasticity of vines and lianas. In S. A. Schnitzer, F. Bongers, R. J. Burnham, & F. E. Putz (Eds.), *Ecology of lianas* (pp. 323–341). Wiley-Blackwell. https://doi.org/10.1002/9781118392409.ch23

Tas, A., Yetisir, H., Denli, N., & Gurcan, K. (2019). Morphological characterisation of bottle gourd (*Lagenaria siceraria* (Molina) Standl.) germplasm and formation of a core collection. *Journal of Agricultural Sciences*, *25*(2), 205–214. https://doi.org/10.15832/ankutbd.429817

Watts, S., & Kariyat, R. (2021). Morphological characterization of trichomes shows enormous variation in shape, density and dimensions across the leaves of 14 *Solanum* species. *AoB Plants*, *13*(6), plab071. https://doi.org/10.1093/aobpla/plab071

Westerband, A. C., Funk, J. L., & Barton, K. E. (2021). Intraspecific trait variation in plants: A renewed focus on its role in ecological processes. *Annals of Botany*, *127*(4), 397–410. https://doi.org/10.1093/aob/mcab011

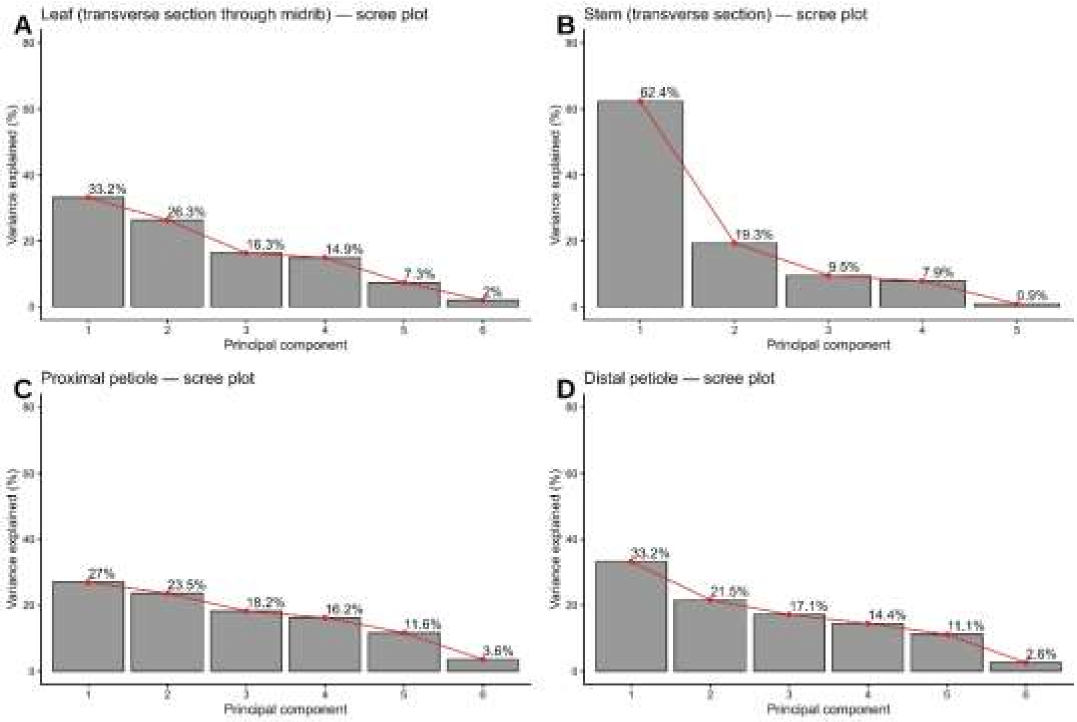

Figure **S1.** Scree plots from per-organ principal component analyses showing the percentage of total variance explained by each component. (A) Leaf. (B) Stem. (C) Proximal petiole. (D) Distal petiole. The line connects values across components.

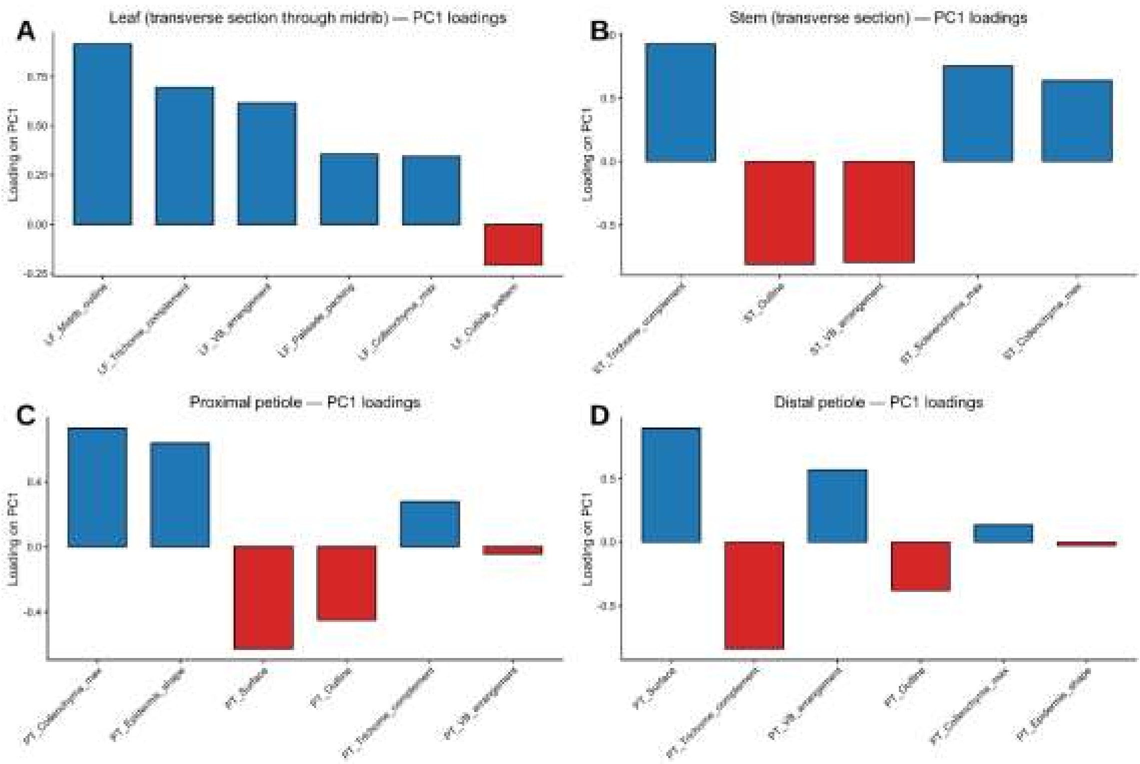

Figure **S2.** PC1 character loadings from each organ analysis, ordered by absolute magnitude. Blue bars indicate positive loadings; red bars indicate negative loadings. (A) Leaf. (B) Stem. (C) Proximal petiole. (D) Distal petiole.

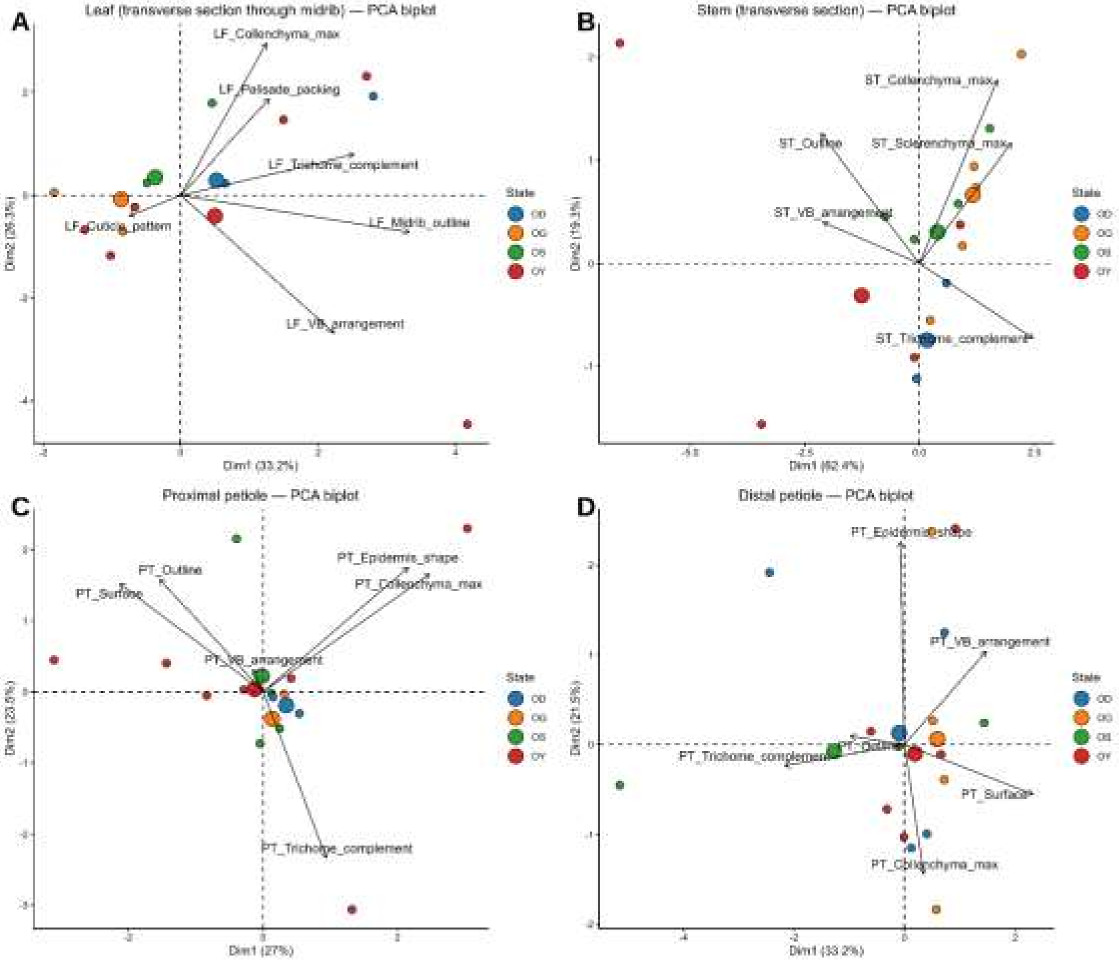

Figure **S3.** PCA biplots of *Lagenaria siceraria* anatomical characters by organ. Accessions are coloured by state of origin: Ondo (blue), Ogun (orange), Osun (green), Oyo (red). (A) Leaf. (B) Stem. (C) Proximal petiole. (D) Distal petiole.