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# Early-Stage Morphological Characterization in Nigerian Snake Gourd (*Trichosanthes cucumerina* L.) Accessions

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## Abstract

Introduction: Morphological characterization provides a practical entry point for conserving and exploiting diversity in neglected crops such as snake gourd. Objective: This study assessed establishment, leaf and seed morphological variation among eight Nigerian snake gourd accessions at the early growth stage. Materials and Methods: Accessions collected from Anambra, Ebonyi, Imo, Lagos, Ogun, Ondo, Osun and Oyo States were evaluated in a randomized complete block design with three blocks. Emergence was analysed by block-adjusted analysis of variance after arcsine-square-root transformation. Leaf shape, colour, lobing, margin, apex, surface and venation were scored, while seed-lot photographs were evaluated qualitatively for colour, outline, margin and surface. Simple-matching dissimilarities were summarized by UPGMA clustering and principal coordinate analysis. Results: Accession effects on emergence were significant ($F_{7,14} = 8.12$; $p = 0.0005$), whereas block effects were not significant ($p = 0.211$). Pooled emergence ranged from 16.7% in Imo-SG to 96.3% in Lagos-SG. Leaves ranged from broad-cordate and slightly or shallowly lobed to palmately and deeply lobed; seed lots differed visibly in brown-tone, oblong outline and degree of marginal scalloping. The first two ordination axes accounted for 64.4% of positive-eigenvalue variation. Conclusion: The accessions possess readily distinguishable early- stage phenotypes useful for germplasm identity, field curation and selection of contrasting parents. Quantitative seed morphometry and multi-environment validation are recommended before inferring genetic control.

Keywords: accession; germplasm; qualitative descriptors; seedling establishment; snake gourd

**Introduction**: Snake gourd (*Trichosanthes cucumerina* L.; Cucurbitaceae), including the cultivated long-fruited form frequently referred to as var. anguina, is an indigenous and underutilized vegetable of tropical production systems. Its local persistence is supported largely by farmer-maintained seed, yet the same decentralised seed system can generate heterogeneous, poorly documented germplasm. Characterization is therefore a prerequisite for avoiding duplicate conservation, maintaining accession identity and identifying divergent materials for pre-breeding. Recent Nigerian evidence has confirmed both appreciable vegetative diversity and molecular differentiation among snake gourd ecotypes, reinforcing the value of combining easily observable descriptors with later molecular validation (Osuagwu *et al*., 2022; Osuagwu *et al.*, 2024). Seed morphology can support seed-lot authentication and reveal visible differences in testa colour, outline, surface and marginal architecture before planting. From the botanical point of view, *T. cucumerina* leaves are described as variable from partly ovate to three - or more-lobed forms, with palmate venation and variable pubescence (Ekeke and Agogbua, 2018). Early field characterization helps identify snake gourd accessions before reproductive traits develop. Nigerian ecotypes differ in vegetative appearance (Osuagwu *et al*., 2022), leaf descriptors alone may miss differences visible in the seed lot or during establishment. Both seed quality and growing conditions affect emergence: Silva *et al*. (2022) reported that physiological maturity and germination temperature influence snake gourd seed performance. Differences in emergence under a common field design therefore provide useful screening information. Characterization of the vegetative parameters as well as the diversity in seeds is needed to guide field curation and the choice of contrasting materials for further study. Multivariate analysis summarize differences across several morphological characters that may be inconspicuous when considered separately. Previous snake gourd studies have used clustering and ordination to describe germplasm variation (Khatun *et al*., 2023; Mahalakshmi *et al*., 2025). The study therefore evaluated eight geographically labelled Nigerian snake gourd accessions at the early growth stage. It compared emergence among accessions under a randomized complete block design, leaf and seed-lot descriptors, and explored their combined phenotypic relationships through clustering and principal coordinate analysis. The absence of adequately characterized snake gourd planting materials limits informed accession choice, systematic conservation and breeding in southeastern Nigeria. Seed lots may differ substantially in emergence, flowering time, fruit form and yield, yet such differences are often undocumented or evaluated without a common design. A field-based characterization using a randomized design, combined with transparent descriptive and multivariate analysis, is therefore needed to identify promising and contrasting materials and to establish a defensible baseline for further trials.

**Materials and Methods:** The experiment was conducted during the 2026 rainy season at the Teaching and Research Farm of the Department of Crop Science and Horticulture, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria. Awka lies approximately at 6°16′ N and 7°07′ E in the humid rainforest-derived agro-ecological zone. The area has a bimodal rainfall pattern with a short mid-season break. The experiment was laid out in a randomized complete block design with eight accessions and three blocks, giving 24 plots. Each plot measured 1.2 m × 2.0 m (2.4 m²). Three seeds were sown per hole at approximately 2.5 cm depth in June 2026. 18 seeds were planted per plot, followed by thinning to nine established plants about two weeks after sowing. Accessions were randomized within each block. The field was cleared manually, marked with rope and pegs and prepared into plots. Cured poultry manure mixed with sawdust was incorporated at a reported rate of 5 t ha−1 before planting. Weeding was carried out manually twice. Bamboo stakes supported the climbing vines. No inorganic fertilizer or supplementary irrigation was applied; plants depended on rainfall. These uniform operations were applied across experimental units.

**Leaf and seed morphological characterization:** Fully expanded, representative leaves were examined at the vegetative stage. Recorded leaf characters were shape, colour, degree of lobing, margin, apex, surface and venation. Terms were applied consistently across accessions: cordate or broad-cordate leaves had a heart-shaped basal outline; palmately lobed leaves displayed pronounced radial divisions; and lobing was classified as slight, shallow, moderate or deep according to the relative incision visible in the leaf blade. Imo-SG additionally showed conspicuous yellow–cream.

**Statistical and multivariate analyses:** Emergence proportions were transformed as $y = \arcsin(\sqrt{p})$ before analysis to stabilize variance. A block-adjusted additive model, $y_{ij} = \mu + A_i + B_j + \varepsilon_{ij}$, was fitted, where $A_i$ is the fixed effect of the ith accession and $B_j$ is the effect of the jth block. Accession significance was tested against the residual mean square at $\alpha = 0.05$. Qualitative leaf and seed states were converted to nominal factor levels. Pairwise simple-matching dissimilarity was the proportion of the seven variable descriptors for which two accessions differed. Invariant leaf margin, apex, surface and venation were summarized descriptively but excluded from distance calculations because they do not distinguish accessions. UPGMA clustering was applied to the dissimilarity matrix. Principal coordinate analysis (PCoA) was performed on the squared dissimilarities; the percentage reported for each axis is relative to the sum of positive eigenvalues. These exploratory outputs describe phenotypic relationships.

**Results: Seedling emergence and establishment**: Emergence differed among accessions after transformation ($F_{7,14} = 8.12$; $p = 0.0005$), while the block effect was not significant ($F_{2,14} = 1.74$; $p = 0.211$; Table 3). Pooled emergence was highest for Lagos-SG (96.3%) and lowest for Imo-SG (16.7%); Anambra-SG also established poorly (37.0%; Table 2 and Figure 1).

**Leaf morphological differentiation:** Leaf shape, colour and lobing differentiated the accessions (Table 4; Plate 2). Anambra-SG, Lagos-SG, Oyo-SG and Ebonyi-SG were broad-cordate, Imo-SG was cordate, and Ogun-SG, Osun-SG and Ondo-SG were palmately and deeply lobed. All accessions shared an entire margin, acute apex, visually smooth surface and palmate venation.

**Seed-lot morphology:** Seed lots differed in apparent colour tone, outline and marginal scalloping (Table 5; Plate 2). Osun-SG showed a dark-brown tone, strongly scalloped margin and very coarse visible surface. Colour classes require validation under calibrated lighting.

**Phenotypic relationships:** The descriptor matrix demonstrated that no two accessions had an identical combination of leaf and seed character states. Lagos-SG and Oyo-SG had the smallest simple-matching dissimilarity (0.286), reflecting their shared broad-cordate, green, moderately lobed leaves and elongate-oblong seeds. Ogun-SG and Ondo-SG formed another recognizable pair through their palmately and deeply lobed leaves, although their leaf and seed colour states differed. Anambra-SG joined the Lagos–Oyo grouping at a modest distance because of its broad-cordate green leaf architecture. Osun-SG remained distinctive because of the combined light-green, deeply lobed leaf and dark, strongly scalloped, very coarse seed phenotype.

**UPGMA and PCoA gave complementary representations of these relationships.** The first two PCoA axes summarized 47.7% and 16.7%, respectively, of the total positive-eigenvalue variation (64.4% cumulatively). Lagos-SG and Oyo-SG occupied closely neighbouring positions; Ogun-SG and Ondo-SG also showed proximity, while Imo-SG, Ebonyi-SG and Osun-SG were separated by their distinctive combinations. The resulting groups are working phenotypic hypotheses suitable for guiding repeat characterization and targeted molecular sampling, rather than definitive genetic clusters.Discussion: Emergence differed significantly among accessions in the common field environment. Six accessions exceeded 85% pooled emergence, whereas Imo-SG and Anambra-SG established poorly. Osuagwu *et al*. (2022) likewise reported germination differences among Nigerian snake gourd ecotypes. Seed maturity and temperature can affect germination: Silva *et al*. (2022) reported physiological maturity at approximately 50 days after anthesis and favourable germination at 30 °C. Seed-lot history was not controlled here, so the emergence differences cannot be attributed solely to genetic factors. Leaf shape, colour and lobing provided visible differences before flowering. Ogun-SG, Ondo-SG and Osun-SG had deeply lobed, palmate leaves, contrasting with the less deeply lobed broad-cordate accessions. Variation in leaf lobing is consistent with the botanical account of Ekeke and Agogbua (2018). The common states of venation, apex, margin and surface were excluded from the dissimilarity analysis because they did not distinguish accessions in this sample. Yellow–cream sectors were observed in an Imo-SG leaf; their cause cannot be determined from the photograph. Seed lots varied in outline and marginal scalloping, but apparent testa colour may also reflect lighting, maturity or storage. Combining leaf and seed characters distinguished accession profiles that shared individual traits. Lagos-SG and Oyo-SG had similar leaves and elongate-oblong seeds, whereas their seed-margin states differed; Ogun-SG and Ondo-SG shared deep palmate lobing but differed in colour and other seed states. Studies by Khatun *et al*. (2023) and Mahalakshmi *et al*. (2025) also illustrate the value of multicharacter descriptions in snake gourd. These groupings can guide the selection of contrasting materials for repeat characterization, but not establish relatedness or predict crossing performance. The state names identify where seed lots were collected, not their genetic origins. Seed exchange may obscure ancestry, and environmental responses may alter the observed characters. ITS variation reported among Nigerian materials by Osuagwu *et a*l. (2024) supports the value of later molecular assessment.

**Conclusion and Recommendations**: The eight Nigerian snake gourd accessions were distinguishable at an early stage through a combination of seedling establishment, leaf architecture and qualitative seed-lot morphology. Lagos-SG, Ogun-SG, Oyo-SG, Osun-SG, Ondo-SG and Ebonyi-SG showed strong establishment, while Imo-SG and Anambra-SG require seed-quality and germination follow-up. Deeply palmately lobed leaves characterized Ogun-SG, Osun-SG and Ondo-SG, whereas Anambra-SG, Lagos-SG, Oyo-SG and Ebonyi-SG were broad-cordate with lesser lobing. Osun-SG was particularly distinctive in the integrated descriptor analysis. These differences are sufficient for preliminary accession identification and selection of phenotypically contrasting snake gourd accessions, but they do not by themselves demonstrate genetic distance.

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**Conflict of Interest:** The author declares no conflict of interest.

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Table 1. Snake gourd accessions included in the early-stage characterization.

| Accession code | Provenance | Material characterized |
|---|---|---|
| Anambra-SG | Anambra State | Seed lot, seedlings and leaves |

| | | |
|---|---|---|
| Ebonyi-SG | Ebonyi State | Seed lot, seedlings and leaves |
| Imo-SG | Imo State | Seed lot, seedlings and leaves |
| Lagos-SG | Lagos State | Seed lot, seedlings and leaves |
| Ogun-SG | Ogun State | Seed lot, seedlings and leaves |
| Ondo-SG | Ondo State | Seed lot, seedlings and leaves |
| Osun-SG | Osun State | Seed lot, seedlings and leaves |
| Oyo-SG | Oyo State | Seed lot, seedlings and leaves |

Table 2. Seedling emergence across three blocks.

| Accession | Planted | Emerged | Block mean ± SE (%) | Pooled (%) |
|---|---|---|---|---|
| Anambra-SG | 54 | 20 | 39.1 ± 23.1 | 37.0 |
| Ebonyi-SG | 54 | 52 | 86.6 ± 1.7 | 86.7 |
| Imo-SG | 54 | 12 | 20.9 ± 7.6 | 16.7 |
| Lagos-SG | 54 | 52 | 96.3 ± 3.7 | 96.3 |
| Ogun-SG | 54 | 50 | 92.6 ± 1.9 | 92.6 |
| Ondo-SG | 54 | 53 | 87.5 ± 4.2 | 88.3 |
| Osun-SG | 54 | 48 | 88.9 ± 6.4 | 88.9 |
| Oyo-SG | 54 | 53 | 91.7 ± 4.2 | 91.7 |

Table 3. RCBD ANOVA of transformed emergence proportions

| Source | Df | SS | MS | F | P |
|---|---|---|---|---|---|
| Accession | 7 | 2.6973 | 0.3853 | 8.12 | 0.0005 |
| Block | 2 | 0.1655 | 0.0827 | 1.74 | 0.2109 |
| Residual | 14 | 0.6646 | 0.0475 | — | — |

Table 4. Diagnostic vegetative leaf descriptors.

| Accession | Leaf shape | Colour | Lobing | Observation |
|---|---|---|---|---|
| Anambra-SG | Broad cordate | Green | Shallow | |
| Ebonyi-SG | Broad cordate | Dark green | Slight | |
| Imo-SG | Cordate | Green | Slight | Yellow–cream sectors |
| Lagos-SG | Broad cordate | Green | Moderate | |
| Ogun-SG | Palmately lobed | Dark green | Deep | |
| Ondo-SG | Palmately lobed | Green | Deep | |
| Osun-SG | Palmately lobed | Light green | Deep | |
| Oyo-SG | Broad cordate | Green | Moderate | |

Table 5. Qualitative seed-lot descriptors

| Accession | Colour | Outline | Margin | Surface |
|---|---|---|---|---|
| Anambra-SG | Light brown | Oblong-rectangular | Irregularly scalloped | Coarse |
| Ebonyi-SG | Light tan | Broadly oblong | Scalloped | Coarse |
| Imo-SG | Pale tan | Oblong | Irregularly scalloped | Coarse |
| Lagos-SG | Medium brown | Elongate oblong | Weakly scalloped | Coarse |
| Ogun-SG | Reddish brown | Elliptic-oblong | Weakly scalloped | Coarse |
| Ondo-SG | Golden brown | Broadly oblong | Scalloped | Coarse |
| Osun-SG | Dark brown | Broadly oblong | Strongly scalloped | Very coarse |
| Oyo-SG | Greyish brown | Elongate oblong | Irregularly scalloped | Coarse |

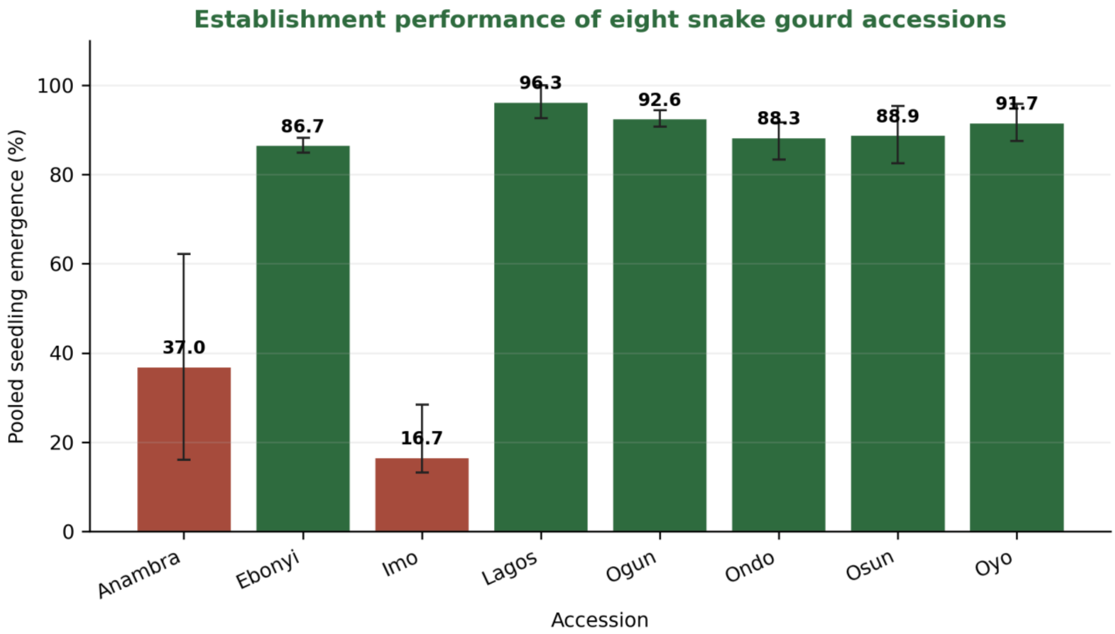


Figure 1. Pooled seedling emergence; error bars show standard error of block percentages.

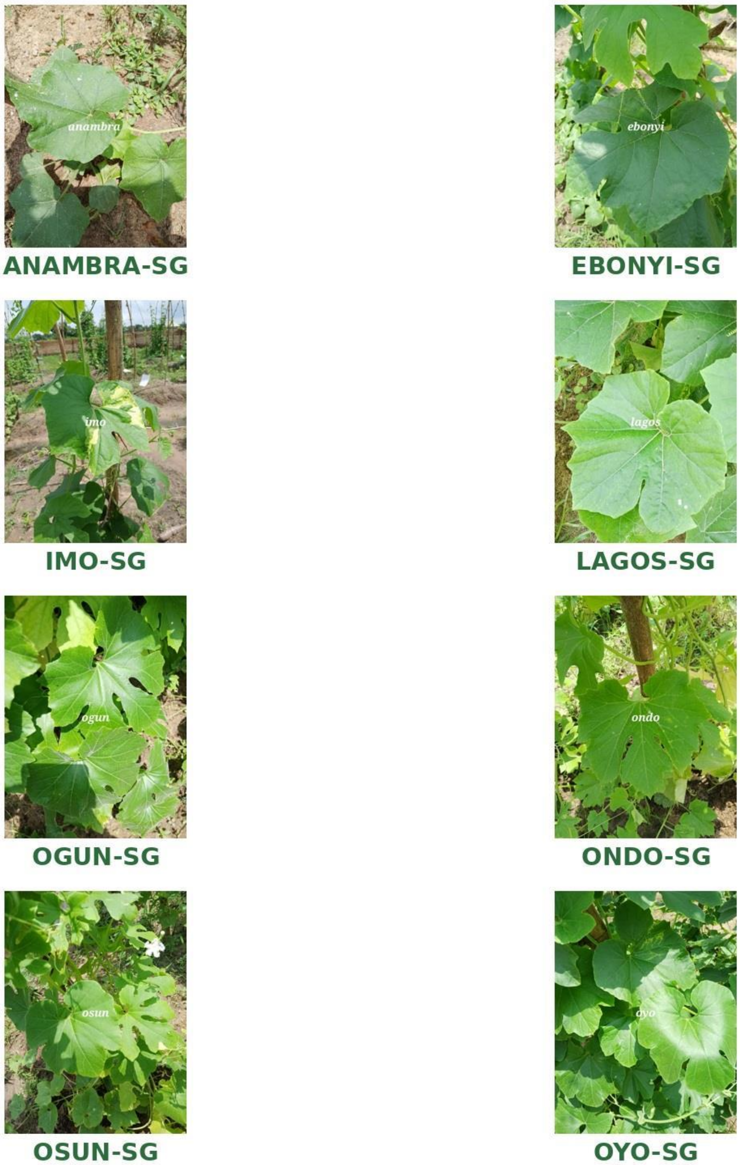


Plate 1. Representative leaf morphology; images are not to common scale.


ANAMBRA-SG


EBONYI-SG


IMO-SG


LAGOS-SG


OGUN-SG


ONDO-SG


OSUN-SG


OYO-SG

Plate 2. Representative seed lots; seed sizes cannot be compared across photographs.

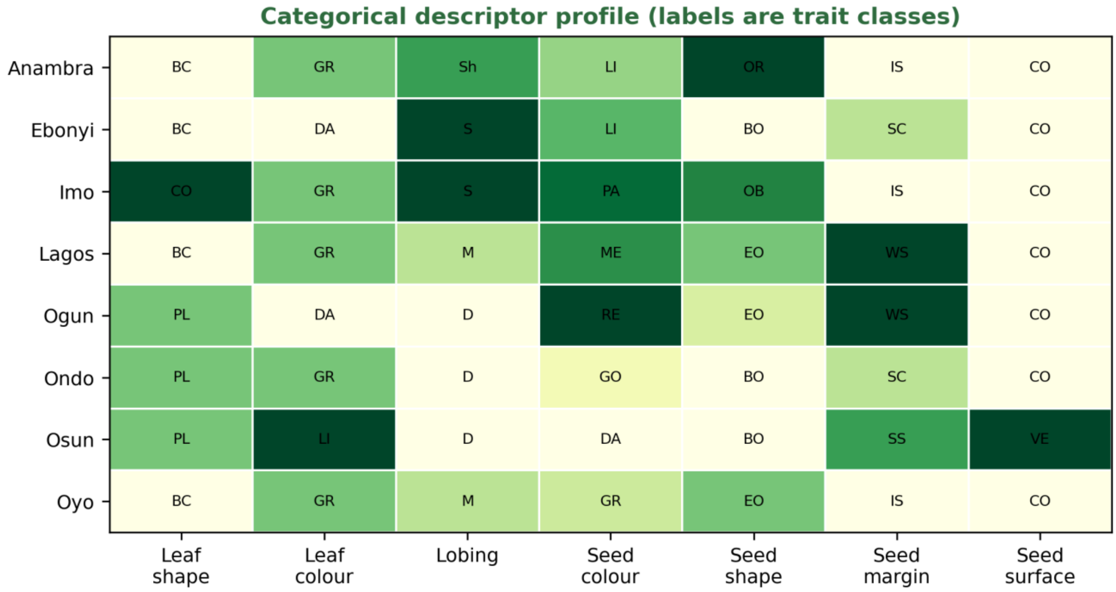


Figure 2. Integrated categorical profile of leaf and seed descriptors.

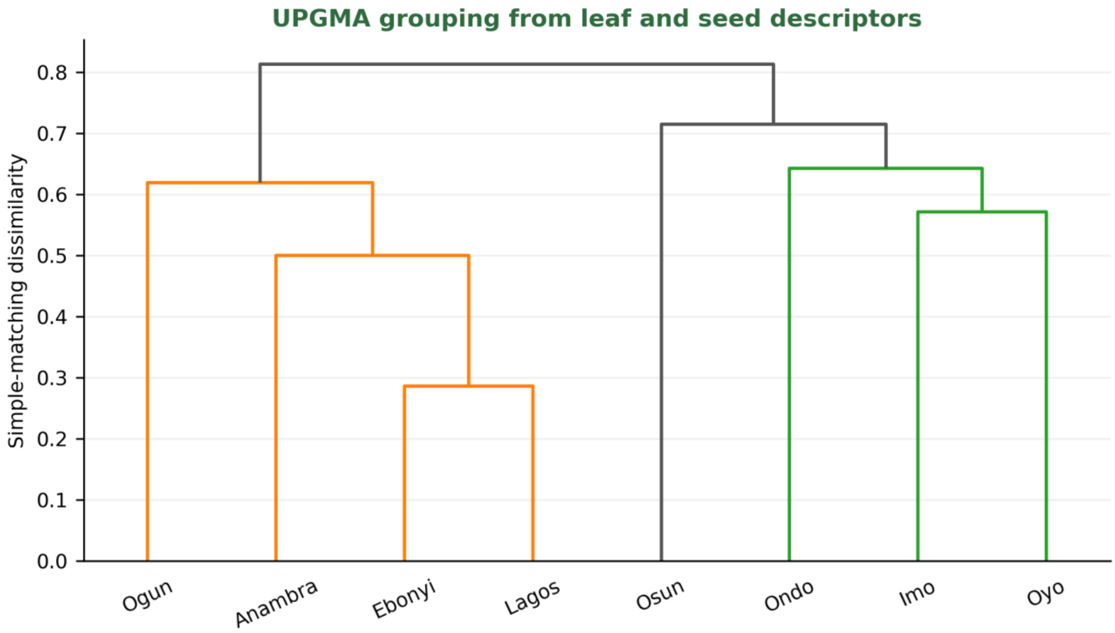


Figure 3. UPGMA dendrogram of seven variable descriptors.

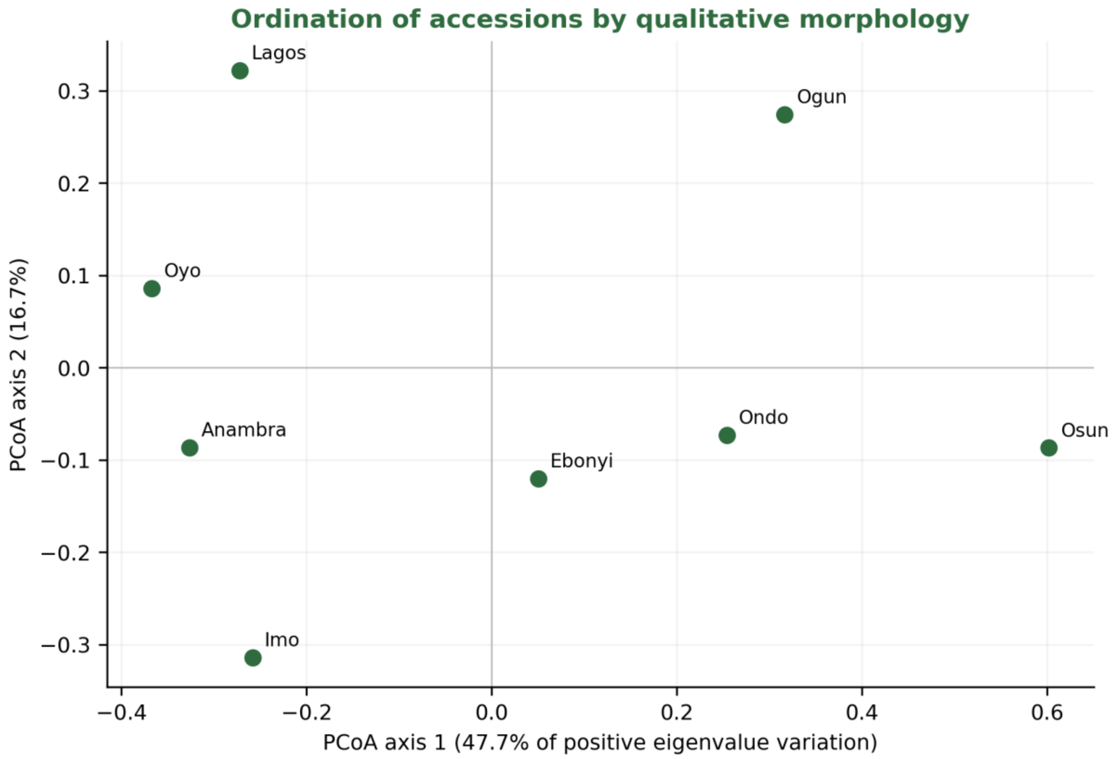


Figure 4. Principal coordinate ordination of qualitative dissimilarities.