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Taxonomic Studies of *Lippia javanica* (Burm F.) Spreng. Complex (*Verbenaceae*) in East African Flora

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Authors' contributions

This work was carried out in collaboration among all authors. Author MWC designed the study, collected data, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author DMM proofread the manuscript and offered guidance in document formatting and data interpretation. Author IM participated in the plant specimen collection, provided technical expertise in collection of taxonomic data, and managed the analyses of the study. Authors MWC, DMM and IM managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

Lippia javanica complex is widely spread in Eastern, Central and Southern Africa; however, there exist a taxonomic conflict in the aforementioned species complex where morphologically similar forms of the species occur in the same habitat. In the Southern Africa region, there are two varieties *L. javanica* var. *javanica* and *L. javanica* var. *whytei* that were formally recognized. Morphological characters of the 63 East African herbarium specimens were sampled. Phenetically, the varieties in the Southern African region overlap in habit and leaf characters. Nevertheless, differences in floral characters such as the number of inflorescence per axil and varying peduncle length form the basis for the morphological distinctness. Multivariate analysis using cluster and

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principal component analysis failed to delimit the infraspecific taxa. Findings from this present study indicated that the variations are not sufficient enough to warrant separation. Thus we proposed that the suspected varieties be synonymized under the earliest name *Lippia javanica*.

Keywords: Lippia javanica; taxonomic conflict; infraspecific taxa; peduncle.

1. INTRODUCTION

Lippia javanica (Burm F.) Spreng more often than not referred to as lemon bush is a multi-branched woody shrub from the tribe Verbenae, family Verbenaceae [1]. The aforementioned species possesses brownish stems that have hairs which are adpressed or short spreading with tubercles and small shinny glands. It is worth noting that the leaves are oppositely decussate or in whorls of three with the blades being lanceolate, elliptic or ovate-lanceaolate and in some circumstances oblong-elliptic. According to [2] the flowers are usually in concoid or oblong spikes; the corolla color ranges from white, yellowish white to greenish white and usually with a yellow throat. *Lippia javanica* nutlets are brown and half-ovoid in shape [3]. This lemon bush is a strong fragrant medicinal indigenous plant that is widely spread in eastern and southern tropical Africa and has also been recorded in the Indian subcontinent [4]. The species is locally abundant in disturbed and rocky hill sites as well as grasslands, open woodlands, fridges of Afromontane forests and riverbanks. According to [1], *L. javanica* is a drought resistant and can grow in a variety of soil types.

The *Verbenaceae* family comprises of approximately 32 genera and 840 species. Phylogenetic relationships within the previously mentioned family demonstrated that the genus *Lippia* and other closely related genera, namely, *Aloysia* Pal´au, *Lantana* L., and *Phyla* Lour were not monophyletic [5]. The genus *Lippia* is composed of approximately 200 species most of which are either shrubs or herbaceous plants, and are widely distributed throughout the tropical, subtropical, and temperate regions of America, Africa, and Asia [6]. The complexion in the taxonomy of *Lippia* genera is not new, as confusions were observed in the past between *Lippia* and *Lantana*. The inflorescences of the two aforementioned species more often than not exhibit similarities [5]. It is notable that both taxa portray spicate heads which become subcapitate during anthesis and elongate into a pedunclulated fruit [5]. Accordingly, several botanists included *Phyla* genera under *Lippia* but [2] divided the former genera basing on the growth form as it is known to being herbaceous. *Phyla* is also characterized by possessing trailing or ascending stems with roots at the node. Furthermore, spikes are longer than broad bearing cuneate-obovate or flabelliform bracts that are not 4-ranked [2]. *Lippia javanica* also seemed morphologically similar to *L. scaberrima* Sond, however, the bracts of the later are shorter than the flowers. *Lippia scaberrima* is multi-stemmed and usually grows to less than 0.5 meters in height unlike *L. javanica*.

Lippia javanica displays morphological differences in the important characters such as the peduncle length and number of inflorescence per axil; this scenario led to the recognition and identification of infraspecific taxa variety *whytei* auct and variety *javanica* in Southern Africa region [5]. Preliminary investigations of the herbarium specimens' revealed morphological variation in the leaf characters; some specimens' possessed opposite-decussate leaves while others the leaves occurred in whorls of three. Differences were also identified in the stem color with some reddish-brown and others gray. Indumetums of the stem were found to possess strigose, strigose-villous, and short stiff tubercle–based hairs with shiny small glands. Furthermore, chemotaxonomic studies indicated existence of chemotypes based on the essential oils which occur profoundly in *L. javanica* [7]. Myrcenone, myrcene and (*E*)- and (*Z*)-tagetenone [8], caryophyllene, linalool, and *p*-cymene [9] whereas geranial, neral. *Cis*-sabinene hydrate and limonene have also been recorded [7,9]. As a result, there is a taxonomic conflict where in East and Central Africa the proposed varieties are informally recognized. There was thus a need to undertake a taxonomic study of *L. javanica* complex based on morphometrics. The proposed variants *Lippia javanica* var. *whytei* and *L. javanica* var. *javanica* overlap in their geographic distribution in East Africa. In the herbarium specimens the two forms have also been hipped together in the same herbarium sheet as they were found growing together [3]. Intermediate forms existed which possessed exceptionally long peduncles, medium length pedicels and the rest almost sessile. Also, the number of inflorescence per axil varied greatly from 1-5 and this phenomenon

proved to be an impediment in the classification of the complex species in the study. These difficulties indicated a need for a delimitation of the specific boundaries between this species complex.

Thus the primary purpose of this study was to use a multivariate technique analysis to discern the species' circumscriptions consistent with that pattern. The paper focused on the following specific taxonomic question: i) Are proposed varieties of *Lippia javanica* complex distinct from each other?

2. MATERIALS AND METHODS

2.1 Plant Material

Specimens of *L. javanica* used in studying morphological features were accessed from the EA Herbarium, Nairobi National Museums. The voucher specimens were from different collectors with their respective numbers, coordinates and other relevant data. Kenyan, Tanzanian, and Ugandan specimens were examined and measurements taken from them by a millimeter hand ruler; a calibrated objective lens of the WILD M3 microscope was employed for tiny features. The type specimen was loaned from the Royal Botanic Gardens, Kew (K), K000379290 and was used in the confirmation if *L. javanica* collections matched the species description together with the Flora of Tropical East Africa-Verbenaceae. The specimens studied in the EA herbarium were according to the extract from the Botanical Research and Herbarium Management Systems (BRAHMS) data base. Specimens collected from the field were incorporated to augment the studies. In each locality visited, five voucher specimens were randomly obtained where the distance from one to the other was a minimum of 100 meters away to avoid collecting related *L. javanica* populations. Label information was recorded i.e. flora area/locality, species details (name, family, habit/description, frequency, and uses), habitat, collector (s) and number (voucher number).Voucher specimens from the field were pressed according to the standard procedures (using plant press and drier) and transported while in the plant press [10]. Upon arrival, they were placed in the drier for three days and were removed for mounting.

2.2 Morphological Data Collection on the *L. javanica* Specimens

Sixty three voucher specimens were studied and only 52 were selected in the final analysis. This was due to immaturity and incompleteness of some characters, i.e. lack of fully open florets and not fully developed inflorescence. Out of the thirty six quantitative characters, only twenty qualified for inclusion in the analysis as they were complete [11]. Eight qualitative characters were used in the description of *L. javanica* characteristics.

The consideration of the specimens included the locality, those with fully mature parts such as flowers, leaves and stems. This was done so as to avoid biases that may arise from developmental plasticity. This also allowed for standardized measurements to be taken for all the specimens thus avoiding bias. The material selected also qualified for inclusion if it matched the descriptions and key characters of *L. javanica* according to [2,3]. The field studies were confined to regions of Kenya where the greatest variability of *L. javanica* are known to occur. The specimens were studied using a WILD M3 dissecting microscope under ×10 and ×40 magnifications. Prior to examining, the floral parts of the preserved specimens were rehydrated in warm water to soften them before dissecting them. Ten measurements of each quantitative character were taken per specimen using a hand ruler calibrated in mm

2.3 Data Analysis

A data set was constructed from the measurements obtained from the accessions that were examined. The matrix comprised of 52 specimens representing the *L. javanica* complex. Multivariate data analyses were applied on the data using STATISTICA Release 7. The following analyses were performed: Cluster analysis (CA) and principal component analysis (PCA). Cluster analysis was performed to show dissimilarities in the operational taxonomic units (OTU's) (specimens in this case) and also to establish if the data grouped them to discrete clusters; the OTUs were clustered based on Unweighted Paired-Group Method Averages (UPGMA) and their degree of similarity measured by L2 dissimilarity measure. Principal component analysis was carried out to further examine the pattern of relationships in the OTU's as well as characters employed [12]. In order to recognize any distinct groupings of similar OTU's, and also separate and re-analyze distinctive groups of OTU's, several runs of PCA were carried out so as to recognize any further patterns of within-group variation [12]. The data set was standardized prior to carrying out the CA

and PCA with the purpose of eliminating characters with large variances [12].

3. RESULTS

3.1 Cluster Analysis for the Morphological Variation

Cluster analysis was performed using the quantitative morphological data. All the *L. javanica* specimens revealed two main clusters A and B at 42.5 L2 dissimilarity measure (Fig. 1). The two clusters were not distinct, thus it was attributed to altitudinal and environmental factors such as climate and soil types. The leaf characters which are usually variable in size were the ones that brought about these variations; and also, cluster analysis has been known to insert a hierarchical structure on the specimens even if the variation is clinal. In fact the supposed varieties were found to cluster together which further confirmed that they were not distinct specimens. A number of specimens from higher altitudes (> 3000 m asl) clustered together.

3.2 Principal Component Analysis (PCA) on Morphological Variation

To further examine the variation in *L. javanica* complex, principal component analysis (PCA) was performed on the data. PCA is oftentimes used to reduce a large set of variables to a small set but no information is lost in the process [12]. Thus, it was carried out to examine the pattern of relationship between OTUs (specimens in this case). The first component axis was derived to encompass the highest percentage of variation among objects. Similarly, the second, the third, and remaining component axes were derived to explain the highest percentage of variation left after derivation of previous axes.

The first two factor coordinates in this PCA explained 42.5% of the variation within the data while the third accounted for further 11.2%. The main factors for separation of these specimens were thus alluded to be altitudinal and the aforementioned environmental factors [13]. Other major factors contributing to variations were the sizes of the leaf (LPLB, LLBA, LWC, and LWNB), (Table 1). Along this factor- plane, the distributions of individuals of the complex were dispersed equally with no signs of morphologically distinct species in the multivariate space. Second factor coordinate similarly exhibited some degree of separation around the multivariate space with no distinct groups as there was an overlap of specimens.

4. DISCUSSION

The Cluster analysis, and Principal component analysis results showed that *L. javanica* consisted of variants, however, not distinct species (Figs. 1 and 2) respectively. Specimens from Kedong which lie in the Great Rift Valley floor were found to possess robust features. The peduncles were exceptionally long implying that the soil and rainfall patterns contributed to this previously mentioned feature. This phenomenon was supported by factor loadings which indicated that variation in leaf sizes contributed more to the clustering (Table 2). This implied that the difference in sizes of the *L. javanica* features was due environmental influence. The weak line of separation of the specimens in the cluster at 42.5 L2 dissimilarity measure was ascribed to environmental and altitudinal factors. According to [13], both regional and altitudinal factors affected the morphological features of various populations, especially those at high altitudes. Rainfall patterns, temperatures, and soil types [13] have been attributed to further aggravate variation. A similarity trend with the *Hemizygia bracteosa* Benth were recorded [11]; accordingly the findings by [14], specimens collected from poor soils with low amount of rainfall had relatively smaller characters as compared to their counterparts from rich soils with even rainfall distribution. It was notable that [13] found out a similar phenomenon in populations of *Cineraria deltoidea* Sond where some specimens had larger capitula in comparison to specimens of the same species which it was ascribed to variations in soil fertility and rainfall patterns [15]. Similarly results in the morphological differentiation of the two forms of *Olinia rochetiana* in South Africa which seemed to reflect adaptations to the micro-climates and ecological conditions in which they occurred [16].

Cluster analysis more often than not is always disadvantageous in that it imposes a hierarchical structure on the data and that the analysis may show distinct clusters even if the variation is clinal (morphological variation in form within a species), as may be seen using ordination techniques [16]. However, morphometric analysis provided a powerful tool for assessing the phenetic relationships among closely related and morphologically similar taxa (14). Cluster analysis (ca) based on the UPGMA method and using average taxonomic distance coefficient as

a dissimilarity coefficient was used as an exploratory method to establish if the data grouped the classes [17]. On the other hand, PCA was recommended for datasets containing quantitative characters and the method has been used to good effect in several taxonomic studies [11].

The variations were also due to the effects of phenetic plasticity that arose from the differences in habitat. This wss due to variation in characters of less taxonomic importance such as leaf characters instead of most important ones like the inflorescence. In concurrence, [14] also reported similar occurrence of specimens of *Vernonia calvoana* to form a continuum of variation with *V. hymenolepsis,* and *V. tolypophora* in PC analysis. *L. javanica* specimens' categorization into *L. javanica* var. *javanica* and *L. javanica* var. *whyte*i based on the peduncle length disparity was not sufficient enough to warrant delimitation [3]; this present study agreed with these latter observations. On the other hand, [1] found out distinct varieties of *L. javanica* in the Southern African region, which contradicted with our present findings. It is worth noting that the morphometric analyses performed failed to reveal variation in the important characters (inflorescence). The dominant variation was in the sizes of the leaf characters and therefore, confirming that the species complex was highly variable but one species.

Lippia javanica complex grows together in many places ranging from disturbed, scrub, and rocky hill sites as well as grasslands, open woodlands, fridges of Afromontane forests and riverbanks. In this study the concept was that a species is the smallest group that is constantly and persistently distinct, and is distinguishable by ordinary means [18]. The term 'by ordinary means,' was taken to mean the visually observable morphological differences. The existence of gaps in the pattern, visually observable phenetic diversity was taken as evidence for reproductive isolation [19]. In this study, the species in question lacked clear morphological discontinuities and thus was not distinct. There was a compelling reason not to separate it; therefore, we proposed that *Lippia javanica* be merged into one morphologically variable species under the valid name *Lippia javanica* (Burm. F) Spreng

Table 1. List of quantitative characters used in the CA and PCA followed by their abbreviations in brackets. All the characters are in mm except flower characters (15-20) which were measured using a calibrated objective lens of WILD M3 dissecting microscope

	Character
	Leaf
1.	Length of the petiole plus the leaf blade (LPLB)
2.	Length of the petiole from the axil to leaf base (LPAB)
3.	Length of the leaf blade from base to apex (LLBA)
4.	Width of leaf blade at the centre (LWC)
5.	Spherical index ratio (SIR)
6.	Leaf width near the apex (LWNA)
	Inflorescence
7.	Length of the flower spike from base to tip (LFBT)
8.	Width of flower spike from base to tip (WFBT)
9.	Number of inflorescence per axil (IPA)
10.	Peduncle length from axil to the base of inflorescence (PLAB)
11.	Length of inflorescence from base to tip (LIBT).
12.	Width of inflorescence at the broadest part (WIB).
	Flower
15.	Length of the corolla tube from base to tip (LCBT)
16.	Length of lower bracts of spike from base to tip (LLBS)
17.	Width of lower bracts of spike at the broadest point (WLBS)
18.	Length of upper bract of spike from base to tip (LUBT)
17.	Width of upper bract of spike at the centre (WUBS)
18.	Length of the corolla from base to tip (LKBT)
19.	Width of the corolla tube at the broadest part (WCBP)
20.	Length of calyx from base to tip (LCBT).

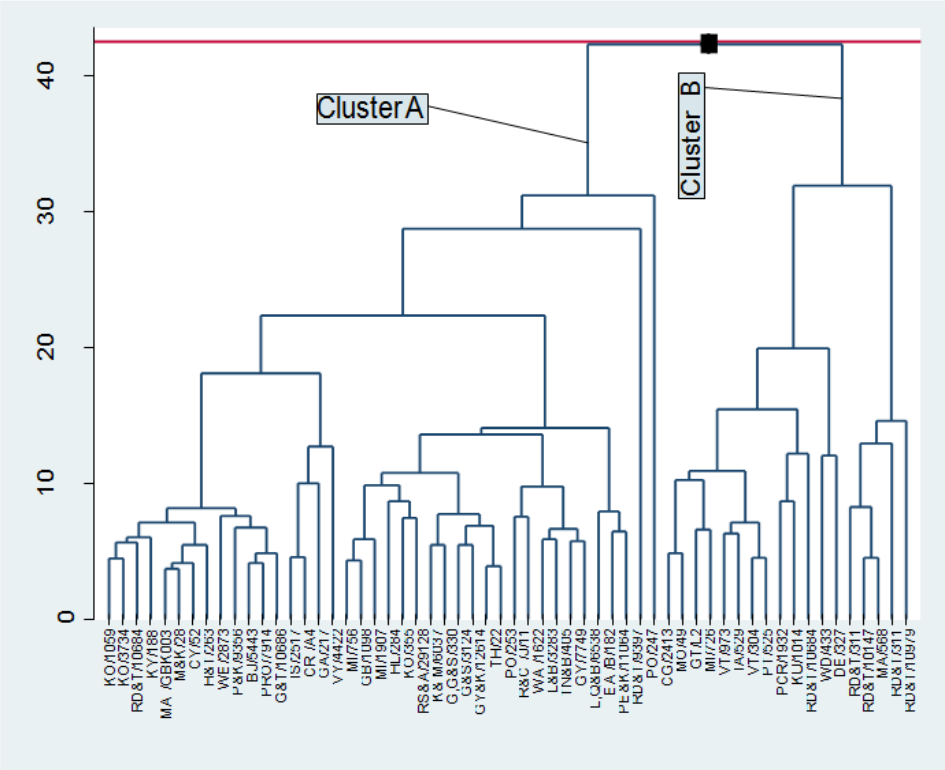

Fig. 1. Dendogram from cluster analysis (UPGMA) of the *L. javanica* specimens. Each OTU has been assigned the first and last letter of the collector and a collector's number. The specimens are according to the BRAHMS data base of the EA herbarium

Table 2. Factor loading on the first 2 factor coordinates for 20 morphological characters of the *L. javanica,* used in the final PCA. Variables with high loadings on each of the principal components are indicated boldfaced

Variable	Factor 1	Factor 2
LPLB	0.915647	-0.196835
LPAB	0.490922	-0.267682
LLBA	0.911725	-0.187797
LWC	0.828519	-0.339856
LWNA	0.739811	-0.431997
LWNB	0.851971	-0.368880
SIR	0.396764	0.208612
IPA	0.349220	0.305307
PLAB	0.388736	0.323096
LIBT	0.485897	0.021058
WIB	0.584259	0.084861
WIC	0.435796	0.464837
WINT	0.321302	-0.041996
LLBS	0.573361	0.444675
WLBS	0.153340	0.245858
LUBT	0.379175	0.555232
WUBS	0.057203	0.506867
LKBT	0.268635	0.442281
LCBT	0.36331	0.471679
WCBP	0.022052	0.697173

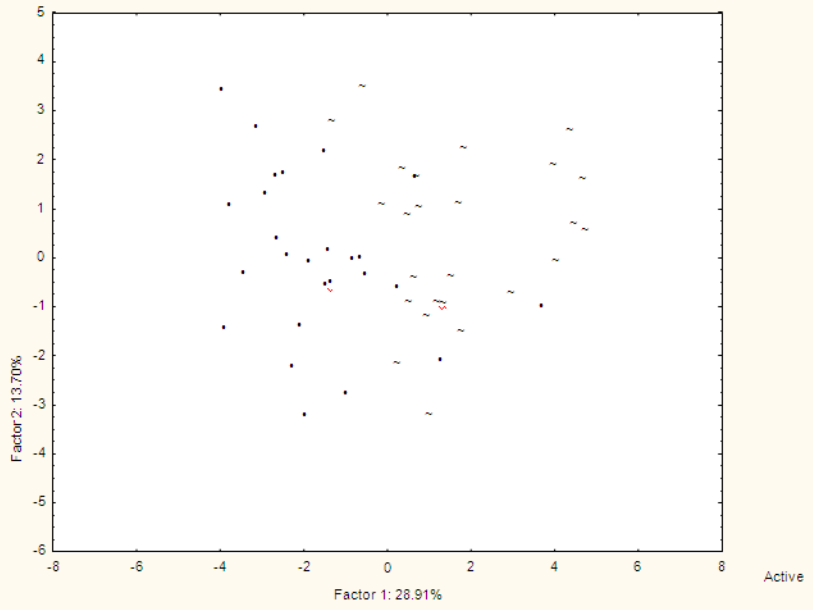

Fig. 2. Scatter plots of the 52 otu's of *I. Javanica* plotted against the first principal component by the second principal component. ~ = *I. Javanica* specimens form1 • = *I. Javanica* specimens second form

5. CONCLUSION

Lippia javanica specimens were found to be one but morphological variable. Therefore, results from this study failed to validate the recognition of the proposed variants var. *javanica* and var. *whytei*. In all the morphometric analysis performed, the two "varieties" mixed to form a coherent group. It is therefore, here recommended that they should be synonymized under the validly published name *L. javanica*.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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