LEONOTIS MENTHIFOLIA IS EITHER, OR NEITHER? #FAKE NEWS? SAD!

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Introduction

The menace of misinformation (**fake news**) affecting society shows little signs of abatement. Even the American horticultural trade has fallen prey due to its embrace of the African ornamental, ***Leonotis menthifolia,*** which is known elsewhere in the world as ***L. ocymifolia***[1]. Understanding its **nomenclature** should be more than a pedantic exercise since *Leonotis* is valued by traditional African herbalists as a medicinal with putative **antimicrobial activity**[2]. Therefore, the horticultural trade could represent one avenue for preservation of important germplasm.

Leonotis is a charismatic, bird-pollinated plant with long, scentless, tubular **orange flowers** distinguished intraspecifically by the number of **annular rings** in the corolla base (fig. 1A). Floral morphology is so uniform that a revision of *Leonotis* reduced dozens of species to synonymy[3]. Molecular work suggests the genus is **polyphyletic** resolving variably within the morphologically-distinct ***Leucas*** and ***Acrotome***[4], both with small, sweet-scented, **white flowers** (fig. 1B). This raises the question of whether *Leonotis* itself is a valid construct. Goldblatt and Manning[5] attempted to address polyphyly by sinking several southern African *Leucas* into *Leonotis*, thereby expanding its morphological definition. We ask, **why not sink *Leonotis* into *Leucas***? **Should *Leonotis menthifolia* even be considered a *Leonotis*?**

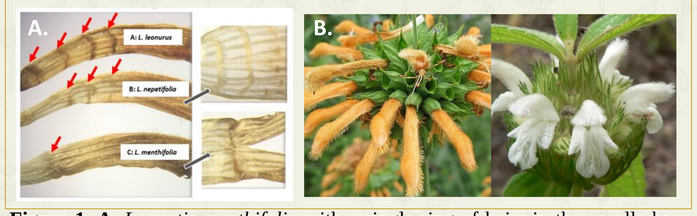

Figure 1. A. *Leonotis menthifolia* with a single ring of hairs in the corolla base, a trait otherwise distinct to *L. ocymifolia*[3]. **B**. *Leonotis* (R) versus *Leucas* (L).

Research Aims:

- Extract cpDNA of *L. menthifolia* for three markers
- Locate 40 lamioid accessions on NCBI
- Construct morphology and molecular data sets of all taxa concerned
- Analyze sets separately with ordination and distance-joining software

Materials and Methods

Molecular work involved extraction of gDNA from ***L. menthifolia*** grown in the Davis greenhouse using a DNeasy Plant Mini Kit[6] with the standard protocol modified for an extended incubation period to 30 min. Sequencing was outsourced to Macrogen[7] for three plastid markers based on Scheen and Albert[4]; *trnL-F* intron, *trnL-F* intergenic spacer, and *RPS16* intron. An additional 40 lamioid accessions (n = 5 *Acrotome*, 8 *Leonotis*, 16 *Leucas*, 9 *Otostegia*, 2 *Phlomis*) were downloaded from NCBI[8]. CLC Sequence Viewer ver. 8.0[6] was used to piece together a *L. menthifolia* contig and force-join individual reads, and for editing, trimming, alignment, and the construction of a clustering cladogram (Neighbor-joining, Euclidean, boot N = 10,000X). No gaps were coded.

Morphological work was conducted by producing an n x n matrix scored as presence/absence data (1/0) for 29 characters gathered from literature[9,10] for 30 core taxa (n = 6 *Acrotome*, 12 *Leonotis*,12 *Leucas*), of which some do not have NCBI records such as the validly published ***Leucas menthifolia***. This was imported into PAST ver. 3.19[11] and analyzed using clustering analysis (Neighbor-joining, Euclidean, boot N = 10,000X) and for ordination analysis (NMS, Euclidean, 2-D).

Results

Since morphological and molecular data sets did not contain identical taxa, data analyses were kept separate instead of opting for a global approach. **Morphology** results using **NMS ordination clearly separated *Leonotis* from *Leucas* and *Acrotome*** with the exception of *Acrotome inflata* (fig. 2A). The same data imported into a **neighbor-joining cladogram** places ***L. menthifolia* as sister to a sister pair of *L. ocymifolia*** (*L. o.* var. *schinzii* sister *to L. o.* var. *ocymifolia*) with 71% bootstrap support (fig. 2B detail). A third variant of *L. o*cymifolia, var. *raineriana*, resolves in a different clade with weak support.

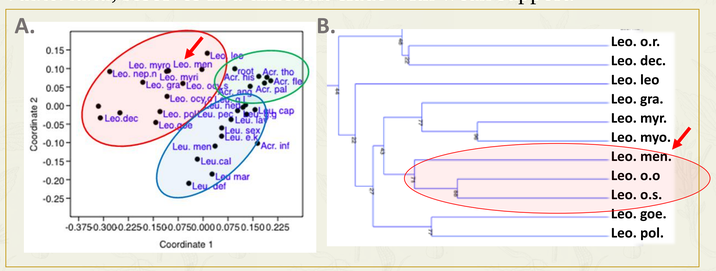

Figure 2. A. Morphology ordination separating *Leonotis* from *Leucas* and *Acrotome*. **B**. Morphology shows *Leonotis menthifolia* as sister to a sister pair of *L. ocyimifolia*.

Sequencing produced a **1781 bp** read similar in length to those returned by Scheen and Albert[4]. **Molecular work also placed *L. menthifolia* as sister to a *L. ocymifolia***, although this time with ***L. o.* var. *raineriana*1** with high bootstrap support (81%), which is sister to a sister pair of *Leucas* (fig 3). *Leonotis ocymifolia* var. *schinzii* instead resolves as sister to a mixed *L. ocymifolia/Leucas* clade, albeit with weak support.

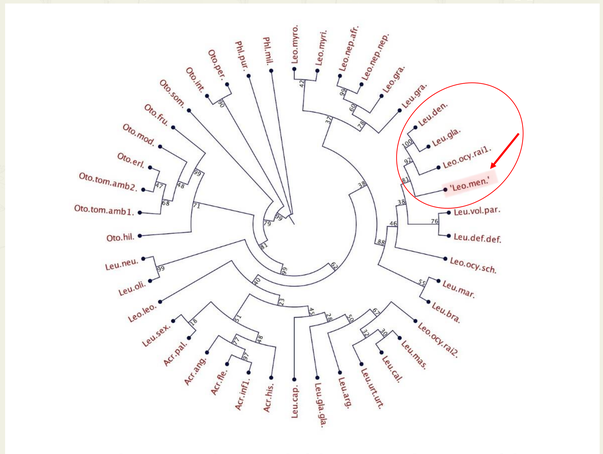

Figure 3. cpDNA placement of *L. menthifolia* in mixed *L. ocymifolia/Leucas clade.*

Discussion

Although proper taxonomic identity may appear arcane in ornamental horticulture, **misidentification** can have repercussions in other sectors such as conservation science, which is problematic during a time of habitat fragmentation and global weather change. Many plants also provide nutraceuticals that can enhance our pharmacopeia since antibiotic-resistance is considered a world-wide threat[12]. Aesthetics aside, *Leonotis* is potentially important since literature suggests they have antimicrobial properties[2]. Based on the International Code for Nomenclature, **it is reasonable to expect the horticulture industry to maintain integrity of their products**, which would also provide a ready mechanism for ***ex situ* conservation** (fig. 4).

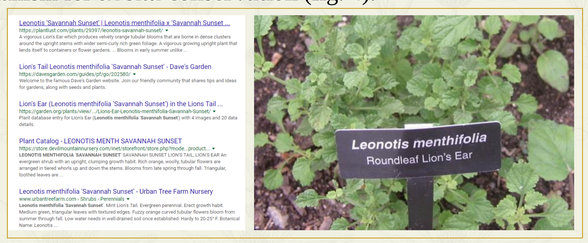

Figure 4. Erroneous 'menthifolia' listings at nurseries and botanic gardens.

Leonotis menthifolia appeared in American horticulture from a West Coast introduction where the genus is hardy. Based on morphology, Henning (unpublished data) stated the taxon keyed out to *L. ocymifolia* in 2001. Our research revisted this assertion in the light of new data that shows ***Leonotis* is polyphyletic**[4], embedding within the **morphologically distinct *Leucas*** and ***Acrotome*.** Not surprisingly, our **morphological evidence** clearly separates the florally-distinct taxa placing ***L. menthifolia* as sister to a clade of *L. ocymifolia*-types**, far-removed from *Leucas menthifolia*. Our **molecular work** places ***L. menthifolia* as sister to an *L. ocymifolia* variety** while reconfirming polyphyly with *Leucas*. Both approaches suggest ***ocymifolia* is the correct epithet** if *Leonotis* is considered a valid entity. However, more work is required since the ***Leonotis*-type flower would have had to arise multiple times within *Leucas***, which suggests simple gene networks might control floral development. In addition, cytology counts and breeding trials would support the case.

In conclusion, ***Leonotis menthifolia*** and ***Leucas menthifolia*** are **separate taxa** with **'menthifolia' a *nomen invalidum* for what should be *L. ocymifolia*.** Deciding if molecular trees are synonymous with species-trees, however, is an argument requiring further investigation.

References

1.SANBI. 2018; 2.Van Wyk. 1997. Med. Pl. S. Afr.; 3. Iwarsson and Harvey. 2003. Kew Bull.; 4. Scheen and Albert. 2009. A.M.B.G.; 5. Goldblatt. 2012. Bothal; 6. Qiagen. 2013; 7. Macrogen. 2018; 8. NCBI. 2017; 9. Codd. 1985. F. Sout. Afr.; 10. Paton. 2009. F. Trop. Afr.; 11. Hammer. 2018; 12. W.H.O. 2018; 13. Henning. Unpub. 2001.

Contributions: Acevedo and Vega did DNA extraction; Acevedo, Camino, and Saldanha conducted morphology work and analysis; Vega edited and analyzed molecular work; poster design and write-up was a group effort.

Acknowledgments: Sincere thanks to Adam McCabe, Christina West, and the Department of Biological Sciences at Lehman-CUNY for technical support.