Leaf Shape Analysis of Closely Related Species in *Passiflora* subgenus *Decaloba* (Passifloraceae)

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Abstract

Passionflower vines (*Passiflora*) have a wide variety of leaf shapes. The research question is whether leaf shape as visualized by a few simple measurements and ratios can be used to identify closely related species. The model system is four species with similar leaves, subgenus *Decaloba* section *Hahniopathanthus*, a monophyletic group occurring in Central America and southern Mexico. Herbarium specimens were measured manually or digitally, ratios computed, and the results plotted on 3-axis graphs. Results show that some of the species can indeed be distinguished using a few simple leaf ratios. This analysis shows promise for future monographic and field-based taxonomic keys.

Introduction

Passion flower vines (*Passiflora*) have the widest variety of leaf shapes of all plants in the world (Ulmer & MacDougal, 2004). The subgenus *Decaloba* is especially variable and includes more than 240 species found mostly in the Neotropics (Krosnick et al., 2013). This subgenus has a variety of trilobed, bilobed, or unlobed leaves. Closely related species tend to have similar leaves. Monographic studies in the genus have included simple descriptions of leaf shape (e.g., degree of lobing) to distinguish certain species, along with traditional use of floral, seed, and other characters. However, most collections and field observations of these vines are of sterile material, without floral or fruit characters available. Thus, field ecologists and biologists cannot identify most collections to species.

Comparing similar leaf shapes in closely related species of passion flower vines requires a more sophisticated descriptor system than simply the amount of lobing. For example, the leaves of species in the section *Hahniopathanthus* are all trilobed, yet differences are noticeable even to the casual observer (Fig. 1). The very few previous studies of leaf shape in *Passiflora* were not used to distinguish closely related species, and because the studies were mathematically highly technical (e.g., Minkowski fractals, Plotze et al. 2005), they are of little use in the field to ecologists. We wondered if a simple method could be developed to identify sterile plants in the field, using only a few easily-made measurements.

Our model system is four species with similar leaves, subgenus *Decaloba* section *Hahniopathanthus*, a monophyletic group of five species occurring in Central America and southern Mexico (Krosnick et al., 2013). The leaves are similar (Fig.1). We chose this small group to determine if leaf shape alone can be used to distinguish the species. The hypothesis is that leaf shape alone can be used to distinguish very closely related species, and that the differences in shape can be described by a few very simple measurements.

Figure 1. Leaves of species of the study group. From left to right, *Passiflora guatemalensis*, *P. hahnii*, *P. membranacea*, and *P. quetzal*.

Materials & Methods

Passiflora specimens were selected from the herbarium of the Missouri Botanical Garden. Only four of the five species of *Hahniopathanthus* were available for observation and measuring: *Passiflora guatemalensis* S. Watson, *P. hahnii* (Fournier) Masters, *P. membranacea* Bentham, and *P. quetzal* MacDougal. One well-pressed leaf was measured from three to ten individual specimens of the four species, resulting in 30 herbarium sheets measured. Specimens were measured directly from specimens with ruler and protractor, or measured from scans using *Image J* software.

Five measurements were made: length of the central vein (CVL), length of one of the lateral veins (LVL), length from the margin to the insertion of the petiole (amount peltate, PEL), width of the leaf (WID), and angle between the lateral veins (LVA) (see Table 1 and Fig. 2). An Excel spreadsheet was used to record data and make simple calculations of the ratios. Data analysis and graphs were made using the software *R* (vers. 3.2.1).

Variable	Description
Central Vein Length - CVL	Distance between vertices A and B
Lateral Vein Length - LVL	Distance between vertices A and C
Peltate Amount - PEL	Distance between vertices A and D
Blade Width - WID	Distance across leaf at widest point - E
Lateral Vein Angle - LVA	Angle between vertices C – A – F

Table 1. List of variables measured in this study. The letters used in the description column match the Figure 2.

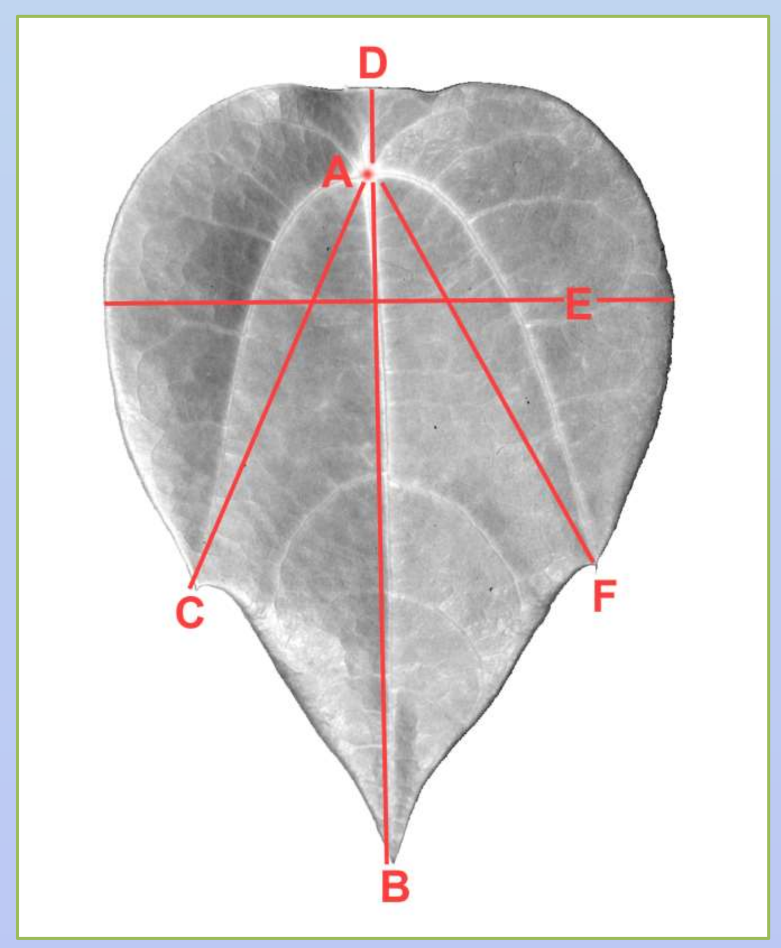

Figure 2. Diagram of how the variables were measured from the leaf blade. Vertex "A" is point of insertion of the petiole. See also Table 1.

Data Structure

For analysis, lengths and widths were changed to ratios except for the lateral vein angles (see Table 2). This was done so we were able to compare general proportions and not raw absolute measurements, thus correcting for differences in overall size, but not shape, of the leaves.

Variables generated	Description
LVL ÷ CVL	Ratio of the lateral vein length divided by central vein length
WID ÷ CVL	Ratio of leaf width divided by central vein length
PEL ÷ (PEL + CVL)	Degree peltate as percentage of total length of leaf (peltate amount plus central vein length)
LVA	Angle between the lateral veins

Table 2. New variables obtained from ratios between the original variables listed in Table 1. These ratios were used for graphing and analysis of the leaf shapes.

Data Analysis

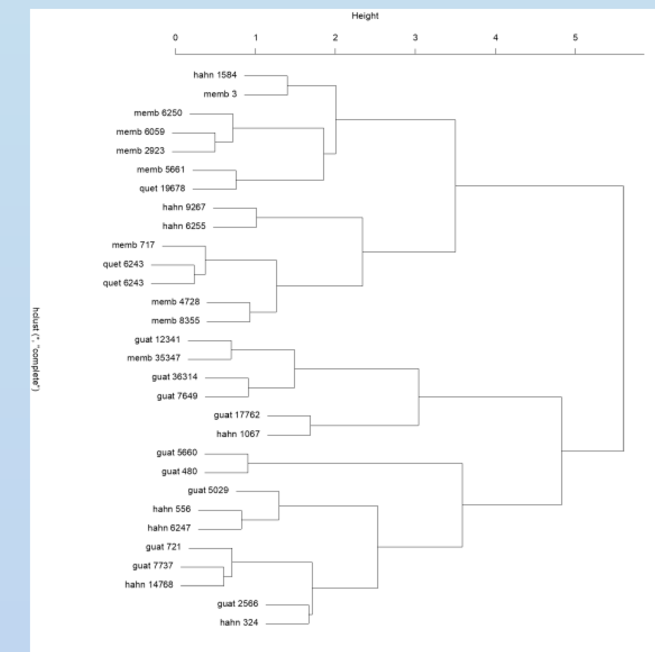

Figure 3. Cluster analysis with four variables: LVL ÷ CVL, WID ÷ CVL, PEL ÷ (PEL + CVL), LVA.

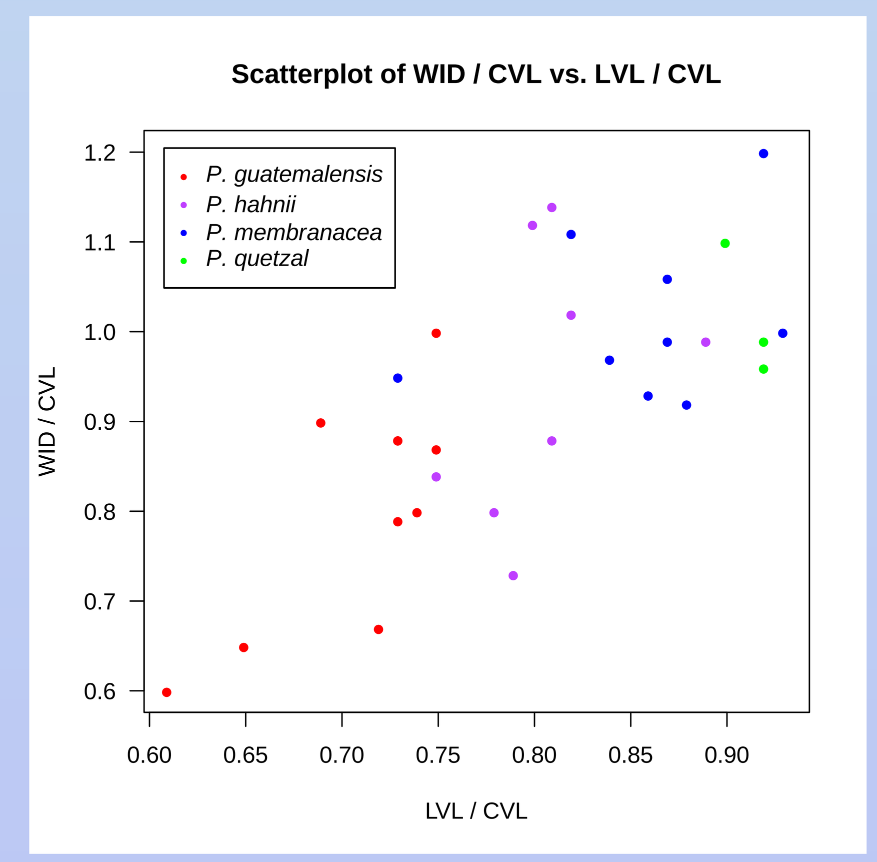

Figure 4. 2-D scatterplot of Leaf Width ÷ Central Vein Length as a function of Lateral Vein Length ÷ Central Vein Length with species indicated in different colors.

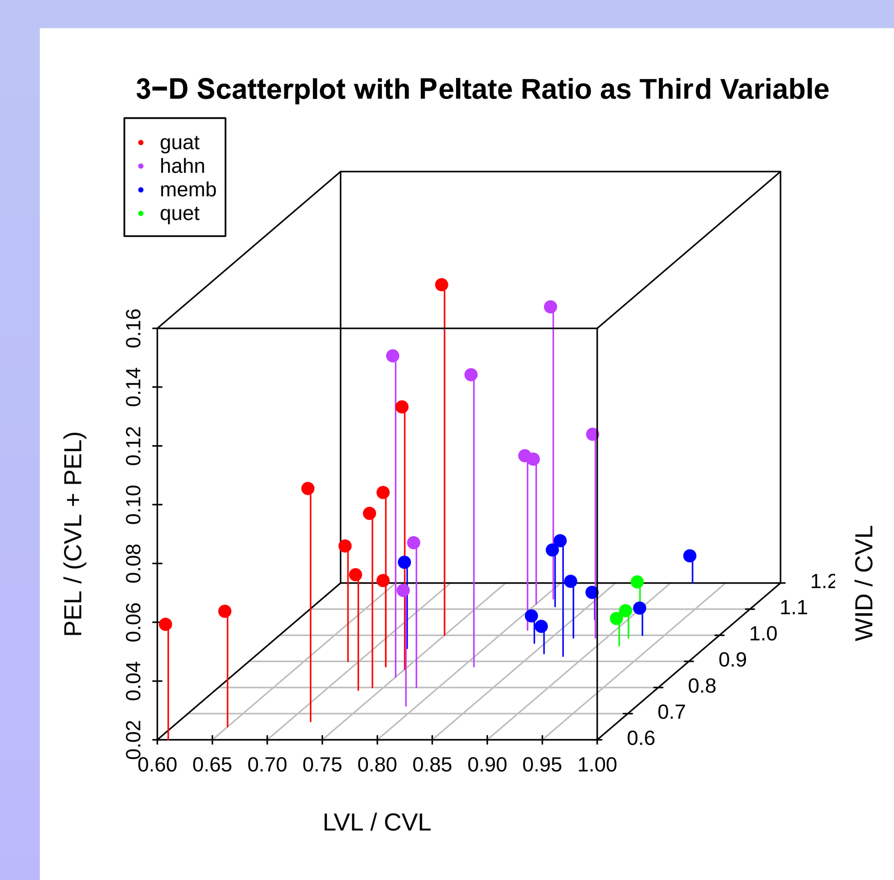

Figure 5. 3-D scatterplot graphing Leaf Width ÷ Central Vein Length as a function of Lateral Vein Length ÷ Central Vein, and adding Degree Peltate as the vertical axis. The species are indicated in different colors.

Results

1. Cluster analysis allows more than three variables to be visualized. Figure 3 shows a clustering using four variables (listed in Table 2). The specimens sorted out partially to species, and the first order dichotomy discerned *P. guatemalensis* from the other species.
2. The 2-dimensional scatterplot (Fig. 4) graphed Leaf Width ÷ Central Vein Length against Lateral Vein Length ÷ Central Vein Length. This clearly shows that *P. guatemalensis* (red) segregates toward the lower left corner of the plot, whereas *P. quetzal* (green) and *P. membranacea* (blue) segregate toward the upper right corner. *Passiflora hahnii* (purple) is more variable and spans most of the center of the graph and overlaps both species groups.
3. The 3-dimensional scatterplot (Fig. 5) graphed the same ratios as the 2-D plot but added a third variable, degree of being peltate or leaf more shield-like (PEL÷ (CVL+PEL)). This helped separate *P. hahnii* (purple) from *P. membranacea* and *P. quetzal*, and in most cases, also distinguished it from *P. guatemalensis*. In Fig. 5, the length of the tail or stem below the data point shows how peltate the leaf is; *P. hahnii* is usually much more peltate than *P. membranacea* (blue), for example.
4. Plots using the vein angles of the lateral veins (LVA) did not give additional resolution in either the 2-D or 3-D plots.

Discussion

Indeed, most of these species can usually be distinguished using a few simple leaf measurements and ratios. We were able to usually identify the species using only four simple measurements made with a ruler. Figure 4 shows *P. guatemalensis* (reds) lower on both the LVL÷CVL (=leaves with center vein long) and WID÷CVL (=leaf longer than wide), while the *P. membranacea* and *P. quetzal* (blues and greens) are higher on both the LVL÷CVL (=leaves with center vein similar to lateral vein) and the WID÷CVL (=leaf more round or square, or even wider than long).

Adding a third variable by measuring if the leaf is slightly or very peltate separates the species enough to give a likely identification (Fig. 5). Field-based taxonomic keys might ask, for example, if the width of the leaf divided by the length of the central vein is 0.9 or less; if so, it is probably *P. guatemalensis*. The identification would be confirmed if that leaf also had a ratio of lateral vein length to central vein length of 0.75 or less. Similarly simple, a leaf peltate 5% (0.05) or less is diagnostic of *P. membranacea* and *P. quetzal*.

We have shown that leaf shape determined by simple measurements can make it simple to identify sterile plants in the field, even leaves that appear similar at first, thus confirming the hypothesis. Leaf shape is highly variable in the passion flower genus, so leaf shape may be a convenient way to recognize species. We look forward to adding more data from *Passiflora* section *Hahniopathanthus*, and investigating variability of the leaves in other sections of the genus.

References

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