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Developmental analysis of Leea guineensis (Leeaceae)

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DEVELOPMENTAL ANALYSIS OF
LEEA GUINEENSIS (LEEACEAE)

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Introduction

Vitaceae is the grape family of flowering plants that is composed of twelve genera of woody plants (Jones et al., 2013). Vitis has been studied extensively for the commercial value of the fruit in the wine and grape production. However, not a lot of research has been done specifically on its basic biology such as leaf development within the family.

Understanding the basic biology of the plant can help with understanding its growth habit and help with improving the overall health of the plant. For example, analyzing flowers and leaves is a good way to draw inferences about developmental similarities and differences across species in the Vitaceae. Although there can be small variations in development between species and even between individual plants, observing them for the patterns in which they share, with those related to them, can be instrumental in solidifying their evolutionary history.

Through this research, I analyzed the morphology and development of *Leea guineensis* (Leeaceae) leaves and flowers. The results of this study will help in future research to compare *Leea guineensis* to two other basal Vitaceae species that belong to clade one; *Rhoicissus digitata* and *Nekemias arborea.* Understanding the results can also help to improve our ability to maintain the health of these species. *Rhoicissus* and *Nekemias* are the most basal of the Vitaceae, making them the prime subjects for comparison with *Leeaceae* due to a lack of derived characteristics that would draw them further from a shared ancestor (Gerrath et al., 2004).

Literature Review

Leaves function as the main photosynthetic organ on a plant. Morphologically, leaves can be divided into three main groups: simple, lobed, and compound. In order to completely understand analysis of leaf development, it is helpful to have some basic knowledge of the different classifications of leaf shape and growth patterns. Even characteristics as simple as these can reveal something about evolutionary connections between different species and different genera.

Simple leaves are those that, even if lobed, are not divided to the midrib. The midrib is the thick vein that starts at the leaf base and stretches to the apex. If an indentation were to reach all the way to the midrib, the leaf would be compound. So long as it does not reach the midrib, it is a simple leaf.

Figure 1: Example of a simple leaf structure.

Compound leaves are those that have several leaflets joined to one stem. There are multiple kinds of compound leaves, including pinnate, bipinnate, and digitally compound. To be digitally compound, the leaflets must originate from one place on the stem like the digits of the hand (Gerrath et al., 2004). A pinnate leaf is one in which small leaflets attach to a rachis which is an extension of the petiole. Therefore, a bipinnate leaf is a pinnate leaf that has leaflets divided into sub-leaflets (Gerrath et al., 1997).

Pinnate leaves can be even or odd. The leaf is odd if it has a single terminal leaflet and even if the apex of the leaf is shared by two leaflets. In some of the more complex plants, multiple different orders of compound leaves can be found to develop together on a single plant.

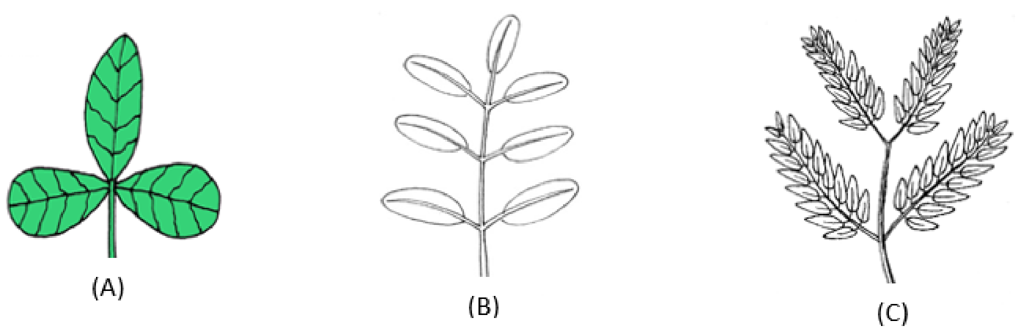

Figure 2: There are many different types of compound leaves, three of which are pictured above. (A) represents a digitally compound leaf. (B) represents an odd pinnate leaf. (C) represents an even, bipinnately compound leaf.

In addition to compound and simple classifications, leaves can also have very different margins. For example, a leaf can have serrated margins, meaning that it has a saw-toothed edge. A leaf can be lobed if the margins have divisions that extend less than halfway toward the base of the leaf. A leaf can also be smooth if it has no serrations or lobes. Recognizing different patterns and types of leaves can help to determine similarities and differences across species of plants.

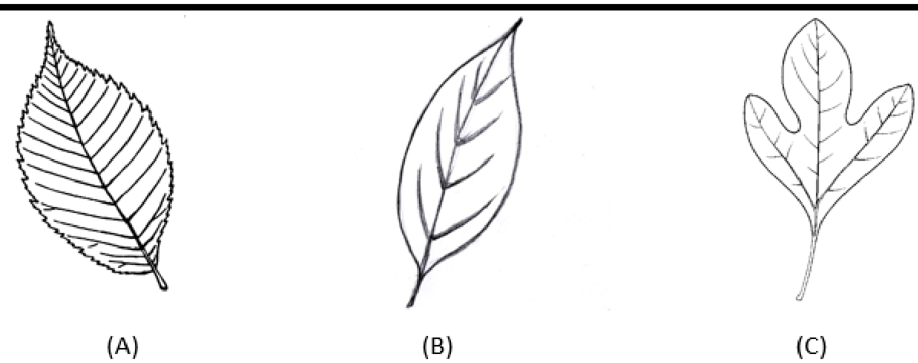

Figure 3: Leaves can have different margins. (A) represents a leaf with serrated margins. (B) represents a leaf with smooth margins. (C) represents a leaf with lobed margins.

The grape family is a large family of plants that consists of fourteen genera and approximately 1000 species (Jones et al., 2004). Members of this family are native to tropical and temperate regions and consist of erect, prostrate, and climbing shrubs. *Vitaceae* is classified as a group of angiosperms, plants that have flowers as well as carpel-enclosed seeds (Jones et al., 2004). Members have a wide range of characteristics; they can have simple or compound leaves as well as bisexual or unisexual flowers.

The relationship between the genera can be found in Figure 4, a proposed phylogenetic tree based on gene sequencing, leaf development, and flower structure (Gerrath et al. 2004).

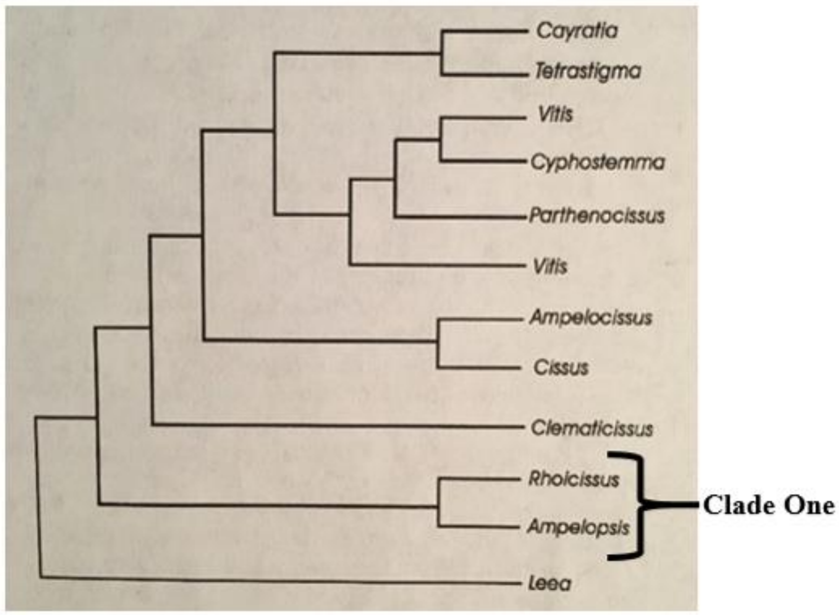

Figure 4: A proposed phylogenetic tree. Clade one consists of two basal groups of *Vitaceae*

It is important to note that the genus *Ampelopsis* has been changed to *Nekemias* (Gerrath et al., 2004). In a study by Gerrath et al. (2004), the authors focused on the derived as well as conserved characteristics of the *Rhoicissus* flower and what those characteristics reveal about the relationship of *Rhoicissus* with other closely related genera in the family. These characteristics included branching of inflorescences, presence of nectar, structure of stamen and gynoecium, as well as orientation of sepals and petals. Through the information compiled about these structures in *Vitaceae,* the authors outlined the basal characteristics that are shared with *Leea.* These include branching of inflorescences in compound dichasia, lack of petal fusion, bisexual flowers, and completion of whorl formation prior to initiation of the next whorl. These characteristics are the only floral characteristics that are known to be shared between basal groups of *Vitaceae* and *Leea.* Therefore, they concluded that *Leeaceae* is related to, but still distant from, the *Vitaceae.*

Leea guineensis:

Leea guineensis, sometimes referred to as *Leea coccinea* in the horticulture trade, is an evergreen shrub that can grow anywhere from six to twenty feet tall (Lacroix et al., 1990). It flourishes in a shady environment with high humidity such as that found in tropical Africa and Asia (Gerrath et al., 1997). It is fast-growing and, with the specific "burgundy" cultivar, has dark green foliage on mature leaves attached to a deep red stem.

Leea guineensis develops monopodially, meaning that it grows upward from one single point with the stem growing longer and leaves being added each year (Gerrath et al., 2007).

Figure 5: *Leea guineensis.*

There are about 70 species contained within the Leea genera. They can be found in Africa, Asia, Australia, and New Guinea. Although it shares many common characteristics with Vitaceae and although some botanists believe it is a member of Vitaceae, Leea contains enough unique characteristics, such as formation of staminodes and secondary septa in the gynoecium, to be classified under its own family, *Leeaceae* (Lacroix et al., 1990).

Rhoicissus:

Rhoicissus digitata is an evergreen vine from the southern portion of Africa. The leaves are smooth, digitally compound, and have distichous phyllotaxy (Gerrath et al., 2004). Having distichous phyllotaxy means that the leaves alternate and grow opposite each other on the stem. The plants crawling growth is assisted by the tendrils present at every node (Gerrath et al., 2004). The tendrils are specialized stems that attach to the plants surroundings for support in climbing plants.

The shoot apical meristem on a plant is the grouping of cells at the tip of a shoot that is capable of producing the new organs and tissues of the plant (Murray et al., 2012). As the shoot apical meristem on *Rhoicissus* develops, it splits into two unequally shaped segments. One of those becomes a new shoot apical meristem, while the other forms either an inflorescence (the flower structure of a plant) or a tendril (Gerrath et al., 2004).

The structural development of each leaf is very uniform, with every leaf at every node being composed of three leaflets in the same digital arrangements (Gerrath et al., 2004). There are some cases in which a leaf will develop five leaflets, but that is not typical. This can be observed by simply looking at the progression of the leaves down the stem and recognizing the uniformity.

When flowering, inflorescences appear at every node. Flowers generally have five unfused, green petals and five stamen accompanied by four ovules (Gerrath et al., 2004). They are bisexual because they contain both stamen (the male reproductive organ in a flower) and a gynoecium (the female reproductive organ in a flower) (Gerrath et al., 2004).

Unlike other genera in the Vitaceae, the flowers of *Rhoicissus* have not been observed to have any derived characteristics (Gerrath et al., 2004). A derived characteristic is one that is not shared with the common ancestor of the clade. Rather, the characteristic formed through generations of evolution and was not originally present before it diverged. This means that, where flower development is concerned, *Rhoicissus* is one of the more basal genera (Gerrath et al., 1997). This makes it a very good candidate for comparison with a group related to Vitaceae such as those found in *Leeaceae.*

Figure 6: *Rhoicissus digitata* is characterized by digitally compound leaves with smooth margins.

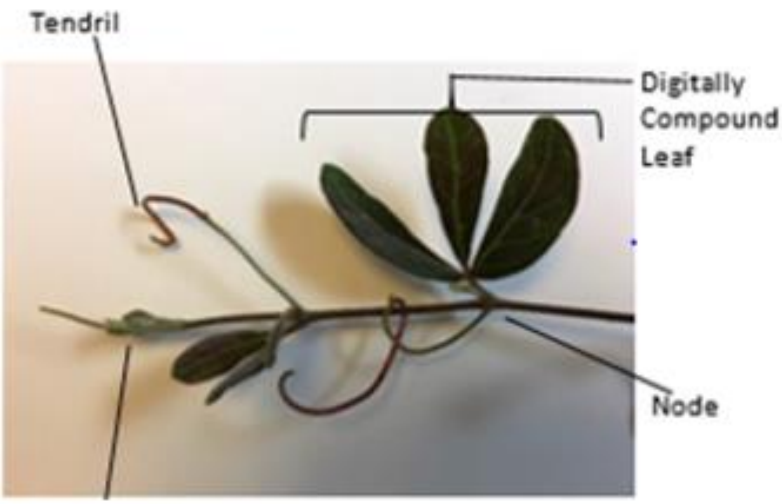

Figure 7: A labeled shoot of *Rhoicissus digitata.*

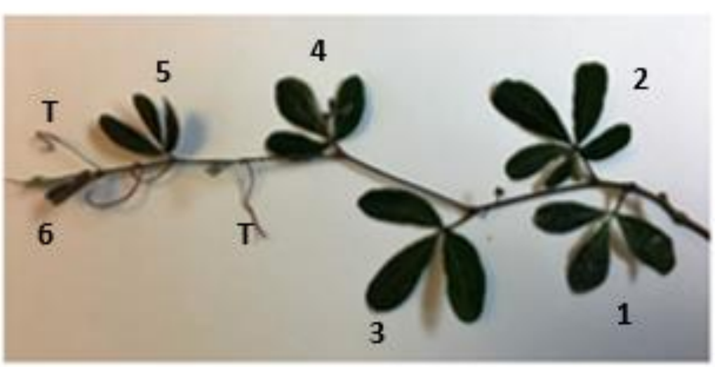

Figure 8: *Rhoicissus digitata* displays distichous phyllotaxy.

Nekemias:

The genera most closely related to *Rhoicissus* that can be used in a comparison with *Leea* is *Nekemias. Nekemias* can be found in the Southeastern United States (Jones et al., 2013). It has serrated and lobed, bipinnately compound leaves that are arranged distichously along the stem. The leaflets are oddly pinnate, so there is one terminal leaflet and several rows of leaflets arranged opposite each other on the petiole.

At the first node, the leaves are often simple or trifoliate. At the second and sometimes third node, the leaflets are simple. Moving further down the leaf, away from the tip, the leaflets become gradually more compound and contain more and more sub-leaflets. As the leaflets emerge further down the petiole (away from the apex), they become pinnate or bipinnate. Like *Rhoicissus, Nekemias* also develops tendrils, but not at every node (Jones et al., 2013).

Figure 9: *Nekemias arborea* leaf.

Figure 10: Node one of *Nekemias arborea* is usually simple or trifoliate.

Figure 11: Leaflets at node two of *Nekemias arborea* remain simple.

Figure 12: Leaflets at node three display a more complex bipinnate structure.

Methodology

Offshoots from *Leea guineensis* were collected from the University of Northern Iowa Botanical Center to examine how leaves develop at each node along the stem. The greenhouses are kept at about 75°F and the plants were checked for water twice a day. Apices were dissected so that the newest developing leaves could be seen. Each shoot was then systematically numbered to create an organized system of analyzing each leaf.

The leaf found furthest from the point where the shoot attached to the rest of the plant was labeled as leaf number one. The next closest leaf was labeled two, the next labeled three, and so on until all leaves on one shoot were labeled. This numbering system was used to compare a leaf on one shoot to a leaf in a correlating position on another shoot. This helps to establish a pattern of leaf development.

The flowers of the plant were also collected for analysis. The flowers were cut from the plant and placed directly in a solution of formalin-acetic acid-EtOH 70% (FAA) fixative and stored for twenty four hours prior to dissection to prevent the tissue from breaking down. Next, the petals were removed using a scalpel to expose the reproductive structures of the plant and the flower was placed under a microscope. Pictures of the flowers were taken alongside a ruler to keep track of the size.

Research Materials:

Photos were taken using a Zeiss AxioCam HRC camera. All microscope work was accomplished using a Zeiss Stereomicroscope.

Results

Leaf Development:

Individual leaves on *Leea* were slightly serrated. Petioles of mature leaves grow perpendicular to the main stem while leaflets grow at an angle slightly tilted toward the apex of the leaf. The phyllotaxy of *Leea*, the way the leaves are arranged in relation to one another on the stem, at first appeared to be distichous, meaning that the leaves formed alternated with each other on opposite sides of the stem. However, when a large shoot was examined, it became apparent that the leaves were actually arranged in a slight but distinct spiral pattern.

An apex on *Leea* begins as a small, round, stipule-covered capsule. The stipule is deep red, tough, and difficult to remove during dissection. The capsule grows flush to the base of the leaf and is located at a node in the crook between the base of the leaf and the stem. Once the developing leaves inside are far enough along developmentally, the stipule opens on the end not connected to the stem. The split in the stipule occurs in a straight line, parallel to the petiole of the leaf it is attached to.

Once free of the stipule, the developing leaves first present as a curled and folded mass resembling a hand curled into a fist. As the leaves grow, they unfurl and spread out. This is a process that takes a couple of weeks to complete. The youngest leaf is contained within the middle of the clump, while the older ones are nearer to the outside. As the leaves age, the surface gradually transitions to a deep green, while the undersides remain red/purple. When they are still young, they are a deep red/purple color.

Figure 13: Young *Leea guineensis* leaves begin as a curled up, stipule-covered mass. They then slowly unfurl and flatten out.

Figure 14: *Leea guineensis* leaflets have slightly serrated margins.

Figure 15: Young *Leea guineensis* leaves are a red color. The color changes as the leaves mature.

The leaflets at the very end of the leaf developed few sub-leaflets while the leaflets closer to the base of the leaf developed multiple sub-leaflets. On each pinnate leaflet, there were always an odd number of leaflets positioned opposite each other on the stem. The leaflets all developed with slightly serrated margins. Sub-leaflets were also found to be odd and opposite each other. The other shoots that were examined corresponded very closely with the findings regarding this numbered shoot.

All mature leaves no matter which shoot they were found on and no matter which node they developed at had a similar pattern when it came to their leaflets. The most distal leaflet was always the most simple of the leaflets. As the leaflets moved further and further away from the tip of the leaf, they became more compound with more sub-leaflets.

The leaflet at the very tip of the leaf was trifoliate and serrated. Node two contained two pinnate leaves opposite each other on the petiole. This pattern is similar to that discussed in *Nekemias.* Both species had a pattern of having simpler leaves at nodes one and two of the shoots. Both of which also have serrated leaves. Leaves two and three are pinnately compound, containing five leaflets. Leaves four and five are still just pinnately compound but contain seven leaves.

It is not until leaves six and seven the leaves took on a bipinnate structure. They each have one terminal leaflet, followed by two rows of simple leaflets, and ending in trifoliate sub-leaflets near the base of the leaf. Once leaf number ten on the shoot was present, no other leaves beyond it became more compound. This implies that there is a limit to how compound a leaf on *Leea* will become. Once they reach a certain level of complexity, they become uniform.

There were three more leaves on the shoot, but none of them were any more compound than leaf number ten.

Figure 16: The leaves on *Leea guineensis* have a pattern of increasing complexity, from simple at the tip of the shoot to complex at the base.

Flowers:

The flowers of *Leea guineensis* grow in domed cymes. Cymes are inflorescences that have many branches which all end in a flower. They are small, waxy, and deep red. Each flower measured out to be about seven millimeters wide from petal to petal and about three millimeters tall from anther to receptacle. Each flower was equipped with five stamen (the combined anther-filament structure) and five unfused, triangle-shaped petals. The stamen are laterally connivent, meaning that the anthers touch each other but they are not fused together. There can be several hundred flowers on a plant at one time which, when pollinated (typically by butterflies, bees, beetles, or flies), are capable of producing a large crop of fruit.

The flowers are actinomorphic, meaning that they have radially symmetry just like the symmetry of a starfish. They have both the male and female reproductive structures contained within one flower, so they are classified as bisexual. The calyx, which is used to protect the reproductive parts of the flower, is bell-shaped.

Figure 17: *Leea guineensis* flowers grow in domed cymes.

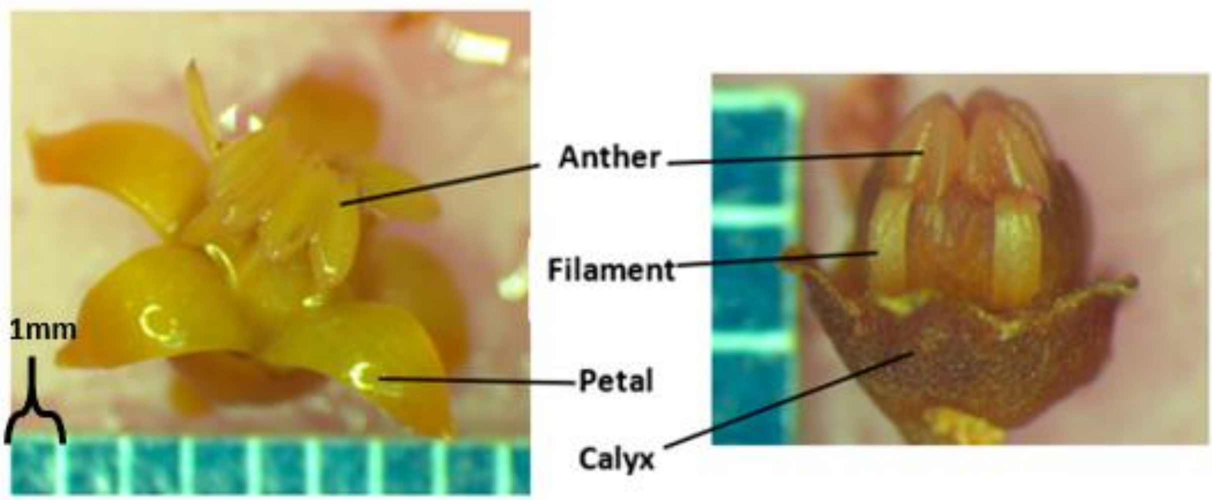

Figure 18: Flowers of *Leea guineensis* are bisexual with connivent stamen, five petals, and radial symmetry.

Discussion and Conclusion

Among Clade One of the *Vitaceae*, *Leea* is the sister to the Vitaceae. Its shrub/tree-like shape and serrated leaves contrast the smooth-leafed vine structure of *Rhoicissus* but have similarities to *Nekemias.* *Leea* has a very complex growth pattern, with a large variety of different leaf types of varying orders of compound structure. The leaflets on *Leea* become increasingly compound the closer they are to the shoot and the more mature they are while remaining simple near the tip of the leaf. *Leea* shares this pattern of leaf formation with *Nekemias.*

The fact that they share such a complex leaf pattern shows that they may be closely related. Two things that should be and will be done in the future to examine this relationship closer will be to analyze the genetic pattern of each species and to look more closely at the SAM. By looking at the genetic makeup, we can establish a pattern for the expression of the *KNOX1* gene (Bharathan, 2002). If the gene is expressed in a similar way in each species, that would serve as further evidence of their relationship (Uchida, 2009).

The advantage of looking at the leaves when they are extremely young is that the young forms can show ancestral traits and structures. Just like examining how embryos of different animal species can look extremely similar and can reveal a lot about possible common ancestry and evolutionary descent, examining very young leaf structures can reveal ancestral connections.

Rhoicissus and *Nekemias* are the two most basal of the *Vitaceae.* This has been established using floral analysis, leaf structure analysis, and genetic testing (Gerrath et al., 2004). *Rhoicissus* has a predictable growth pattern, with apices and tendrils forming at every node and every leaf structured in a trifoliate, digitally compound way. Despite this, there are similarities between these groups.

They both have small, bisexual flowers that form in clusters, cymes, that are complexly branched (Gerrath et al., 2004). Also, the petals of all of the flowers in each species are unfused. However, the laterally connivent stamen are unique to *Leea,* supporting the hypothesis that *Leea* is related to, but not part of, the *Vitaceae.*

This information on *Leea guineensis* will be used in future research to compare it to some if its closest relatives. Establishing similarities and differences between the members of Clade One will help to uncover how the members of this family are related to one another as well as when and how they diverged from one another. Because there can be so many small variations within species that do not necessarily mean anything phylogenetically, it will be important to get a more inclusive and all-encompassing view of each species.

For this reason, future research will require a full, ontogenic and heteroblastic analysis of the development of each species. Ontogenic analysis is when something is studied from its earliest development to maturity. This full analysis will better highlight the similarities and differences between the species and will create a more solid picture of how they may be related to one another evolutionarily. This solid picture can be used to improve the health of the plant as well as to uncover possible uses for the plant that have not yet been considered.

Figure 19: *Leea guineensis* and *Nekemias arborea* share serrated leaf margins and pattern of compound structure. They bear fewer similarities to *Rhoicissus digitata.*

Terms and Definitions

Actinomorphic—having radial symmetry.

Angiosperm—plants that have flowers as well as carpel-enclosed seeds.

Bipinnate—a pinnate leaf that has leaflets divided into sub-leaflets.

Calyx—protective structure formed by the sepals of a flower.

Connivent—coming into contact but remaining unfused.

Conserved characteristics—traits retained throughout evolution.

Cultivar—plant variety produced by selective breeding.

Cyme—inflorescences that have many branches which all end in a flower.

Derived characteristics—traits that are not shared with the common ancestor of the clade.

Digitally compound-- leaflets originate from one place on the stem like the digits of the hand

Distichous—leaves alternate and grow opposite each other on the stem.

Gynoecium—the female reproductive organ in the flower.

Inflorescence—the flower structure of a plant.

Midrib—the thick vein that starts at the leaf base and stretches to the apex.

Monopodial—growth upward from one single point with the stem growing longer and leaves being added each year.

Node—locations along the stem capable of growing new leaves.

Ontogenic—study from earliest development to maturity.

Petiole—joins the leaf to the stem.

Phyllotaxy—the arrangement of leaves along the stem.

Pinnate—leaf structure in which small leaflets attach to a rachis.

Rachis—extension of the petiole.

Shoot apical meristem-- the grouping of cells at the tip of a shoot that is capable of producing the new organs and tissues of the plant.

Stamen—the male reproductive organ in the flower.

Stipule—leaf-like appendages that at first protect the developing leaves and then reside on either side of the base of the leaf.

Tendril—specialized stems that attach to the plant's surroundings for support in climbing plants.

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