



Article

Trichomes' Micromorphology and Their Evolution in Selected Species of *Causonis* (Vitaceae)

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Abstract: The Vitaceae genus *Causonis* is found in tropical to temperate climates from Asia to Australia, including the Pacific Islands. Rafinesque established the genus in 1930; however, Süssenguth classified it under *Cayratia* as a sect. *Discypharia* in 1953. The genus was resurrected in 2013 using morphological and genetic evidence. We herein provided insight into the diversity of trichomes' micromorphology of selected species of this recently reinstated genus for taxonomical implication. Simple trichomes, representing non-glandular and unbranched trichomes, are only found in *Causonis*. Trichomes vary from straight, curved, hooked, appressed, pilose, to villous in different parts like branchlets, abaxial leaf surface, and adaxial leaf surface in different species. They also vary in the same plant from the young stage to the mature stage. Most species are pubescent when young, but a few species become nearly glabrous when they are mature. Significant variations can be observed in trichomes' length between the species. Principal component analysis (PCA), based on the micromorphological traits, was carried out for the species delimitation. In Mesquite, ancestral character state reconstruction was used to examine evolutionary trends for trichomes on three different surfaces. The glabrous to sparsely pubescent state found on the branchlets and both leaf surfaces were found to be the ancestral state and, on the branchlets and both leaf surfaces, the villous hairs state was the derived state in the genus. The identification of *Causonis* species is greatly aided by trichomes morphology. Therefore, similar studies should be conducted on other Vitaceae genera to reveal the variety of trichomes found in the family.

Keywords: character evolution; hairs; identification; morphology; stereomicroscopy



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1. Introduction

Vitaceae is a widely known family of commercial significance owing to grapes, wines, and raisins [1]. In addition, the family also has some plants with ornamental values like *Cissus verticillata* (L.) Nicolson & C.E. Jarvis, *Parthenocissus quinquefolia* (L.) Planch., and *P. tricuspidata* (Siebold & Zuccarini) Planch. [2]. It consists of 16 genera and ca. 950 species [3]. A wide range of research is being carried out in the family from a general morphological description of new taxa (species or genus) and already described taxa or re-circumscription of taxa to different molecular level works such as phylogenetics, character evolution, and biogeography. A large number of molecular works performed on the family include phylogenetic analysis using selected chloroplast and/or nuclear markers [4–11]. With the advent of new technologies, the paradigm in the Vitaceae research has been slowly shifting from phylogenetic study to phylogenomic study to reveal long-standing phylogenetic questions like resolving the non-monophyly of taxa because of gene duplication or loss, hybridization, introgression, or incomplete lineage sorting [11–13].

Causonis Raf. is a recently segregated genus of Vitaceae. Wen et al. [14] resurrected the genus *Causonis* from the genus *Cayratia* Juss. (hereafter *Cayratia* s.l.) based on both molecular phylogeny and morphological evidence. Morphologically, *Causonis* do not have ventral infolds covered with a distinct membrane in seeds, while *Cayratia* s.s. have ventral

infolds covered with a distinct membrane. Rafinesque created the genus in 1830 [15], although Gagnepain [16] considered them to be *Cayratia* species. Suessenguth [17] and Latiff [18] classified them as members of the *Cayratia* sect. of *Discypharia* Suess. Later, sect. *Discypharia* was treated as subg. *Discypharia* (Suess.) C.L.Li of *Cayratia* by Li [19], who was then followed by Chen, et al. [20]. Subg. *Discypharia* has a non-articulate inflorescence axis, without bracts on the inflorescence axis and ventral infolds without a distinct membrane covering, versus the articulate inflorescence axis, with bracts on the inflorescence axis and ventral infolds covered with a distinct membrane in subg. *Cayratia*.

Two species of subg. *Discypharia*, *Cayratia oligocarpa* (H. Lév. & Vaniot) Gagnep. and *C. albifolia* C.L.Li, were transferred to a new genus *Pseudocayratia* J.Wen, L.M.Lu & Z.D.Chen [21] based on the morphology in particular seed characters. Three Australian species were moved to *Causonis* by Jackes [22], namely, *C. clematidea* (F.Muell.) Jackes, *C. eurytnema* (B.L.Burtt) Jackes, and *C. maritima* (Jackes) Jackes, based on endosperm shape in the seeds' cross section. The molecular phylogeny of any species requires morphological support for its distinct taxa confirmation. The identification of the genus and species of the Vitaceae has been facilitated by the use of seeds [3,10,23–25]. Gerrath et al. [26] revealed interesting findings regarding tendrils and the position of inflorescences in the Vitaceae. Trichomes, a vegetative character, are very useful and are often used in the keys for the identification of species within the genus [20,21,27]. Nonetheless, it has been poorly studied in detail in any particular genus of the family, except genus *Vitis* L. by Ma et al. [2,28].

Vitaceae is one of the families with problems in the identification of its taxa because of huge variations in its vegetative characters like leaves' architecture, tendrils' furcation, veinlets' number, veinlets' position, and sometimes trichomes [11,20,27]. These morphological characters vary from the young stage to the mature stage in the same plant. Taxa having reproductive parts as diagnostic characters are often difficult to identify in the absence of flowers or seeds. The vegetative parts like branchlets and leaves are generally easily accessible irrespective of the season or stage of growth. The identification of plants can be greatly aided by the diagnosis of plants based on these two morphological characters in the absence of floral parts or seeds, though it is not applicable every time. Trichomes can thus be incredibly helpful in identifying taxa within the Vitaceae. In addition, some *Causonis* species, such as *C. japonica* and *C. trifolia*, are highly medicinal and used to treat a variety of maladies [11]. As a result, they can be suggested for horticulture. Therefore, with an aim to unveil trichomes' diversity in different species of this horticulturally important *Causonis*, we herein (1) study different types of trichomes on stems, abaxial, and adaxial leaf surfaces for taxonomic implication in *Causonis*; (2) investigate quantitative features of trichomes on stems, abaxial, and adaxial leaf surfaces in the genus; and (3) trace evolutionary trends of trichomes on stems, abaxial, and adaxial leaf surfaces in the genus.

2. Materials and Methods

2.1. Taxon Sampling and Identification

In this study, 14 specimens representing 12 taxa of *Causonis* were studied. All of the specimens sampled were recently collected from different distribution regions of the genus, except *C. ciliifera* (Merr.) G.Parmar & L.M.Lu and *C. tenuifolia* (Wight & Arn.) G.Parmar & L.M.Lu, which were observed from the deposited specimens at the National Herbarium (PE), Institute of Botany, Chinese Academy of Sciences, Beijing. The plants were identified following Chen, et al. [20] and Jackes [27]. One additional specimen each of *C. trifolia* (L.) Mabb. & J.Wen and *C. timoriensis* var. *mekongensis* (C.Y.Wu ex W.T.Wang) G.Parmar & L.M.Lu were included in this study to show the variation in trichomes within the species. The voucher information of all studied samples is included in Table 1.

2.2. Microscopic Investigation

Trichomes' diversity was examined from all of the specimens at PE. All specimens were examined for variations in trichome morphology on branchlets, abaxial leaf surfaces, and adaxial leaf surfaces using a stereomicroscope with a Leica DVM6 camera. Images of

trichomes on different parts were taken using a scale. The terminology used for describing trichomes follows Chen et al. [20] and Jackes [27].

Table 1. Voucher information of samples used in this study.

Taxon	Voucher No.	Locality
<i>Causonis ciliifera</i> (Merr.) G.Parmar & L.M.Lu	PE00686401 (PE)	China, Hainan, Jianfengling
<i>Causonis clematidea</i> (F.Muell.) Jackes	PE01966347 (PE)	Australia, Queensland, Cattle creek road
<i>Causonis corniculata</i> (Benth.) J.Wen & L.M.Lu	CPG09774 (PE)	China, Taiwan, Nantou
<i>Causonis daliensis</i> (C.L.Li) G.Parmar & L.M.Lu	VN2014116 (PE)	Vietnam, Lam Dong, Bidoup-Nui Ba
<i>Causonis fugongensis</i> (C.L.Li) G.Parmar & L.M.Lu	CPG33017 (PE)	China, Yunnan, Ruili
<i>Causonis japonica</i> (Thunb.) Raf. var. <i>japonica</i>	CPG11331 (PE)	China, Yunnan, Hekou
<i>Causonis japonica</i> var. <i>pseudotrifolia</i> (W.T.Wang) G.Parmar & J.Wen	CPG20403 (PE)	China, Sichuan, Guangyuan
<i>Causonis maritima</i> (Jackes) Jackes	AU020 (PE)	Australia, Queensland, Cairns
<i>Causonis mollis</i> (Wall. ex M.A.Lawson) G.Parmar & J.Wen	LA41 (PE)	Laos, Champasak, Pakse
<i>Causonis tenuifolia</i> (Wight & Arn.) G.Parmar & L.M.Lu.	CPG38698 (PE)	India, Kerala, Mannarkad
<i>Causonis timoriensis</i> var. <i>mekongensis</i> (C.Y.Wu ex W.T.Wang) G.Parmar & L.M.Lu	CPG32937 (PE)	China, Yunnan, Yingjiang
<i>Causonis timoriensis</i> var. <i>mekongensis</i> (C.Y.Wu ex W.T.Wang) C.L.Li	CPG18926 (PE)	China, Yunnan, Ruili
<i>Causonis trifolia</i> (L.) Mabb. & J.Wen	LA17 (PE)	Laos, Luang Namtha, Muang Sing
<i>Causonis trifolia</i> (L.) Mabb. & J.Wen	CPG23885 (PE)	Vietnam, Ninh Thuan, Ca Na

2.3. Quantitative Study

Trichomes' lengths were measured for mature branchlets, abaxial leaf surfaces, and adaxial leaf surfaces, including the shortest and longest trichomes on the surface, for each species, if present. On each of the three parts of each species, six distinct trichomes were measured including the shortest and longest trichomes, and the standard deviations were calculated. Species lacking trichomes on specific parts were marked as absent. Trichomes' length of *C. trifolia* (accession number LA17) and *C. timoriensis* var. *mekongensis* (accession number CPG32937) were only recorded for the respective taxa because other species were also represented by only one accession in this study. Accessions of *C. ciliifera* and *C. tenuifolia* were studied for the types of trichomes from old collections, but they could not be studied for trichomes' length because of the limited accessibility of those samples.

2.4. Ancestral Character State Reconstruction

For the reconstruction of ancestral character states, trichome morphological characteristics based on the position on three different plant parts, such as trichomes on branchlets, trichomes on abaxial leaf surface, and trichomes on adaxial leaf surface, were chosen. The evolution of characters was reconstructed in Mesquite 3.61 using the chloroplast dataset. The "Trace Character History" and the Markov k-state one-parameter (Mk1) evolutionary model were employed in a maximum likelihood (ML) approach for character state reconstruction [29].

3. Results

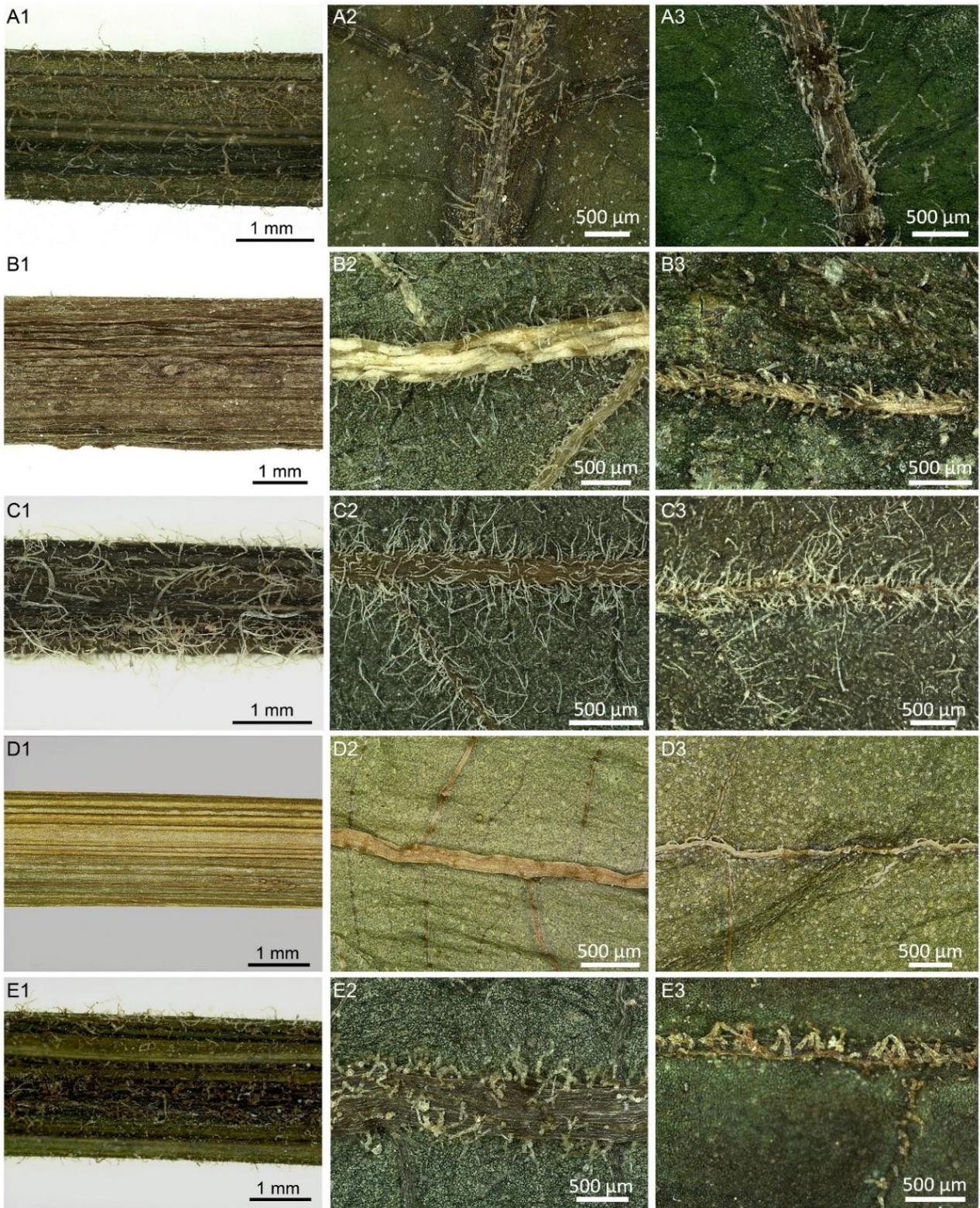
3.1. Microscopic Investigation

The study performed on 14 different specimens of *Causonis* revealed two distinct types of trichomes: normal hairs and villous hairs. Pilose hairs, appressed hairs, hooked hairs, and curved hairs were also observed, but were very rare in occurrence (Figure 1). Specimens were found to be almost glabrous on the branchlets in *C. maritima*, *C. corniculata* (Benth.) J.Wen & L.M.Lu, *C. tenuifolia*, *C. japonica* (Thunb.) Raf. var. *japonica*, and *C. japonica* var. *pseudotrifolia* (W.T.Wang) G.Parmar & J.Wen. *Causonis timoriensis* var. *mekongensis* was found to be sparsely pubescent with simple hairs. Puberulent hairs were only found on the branchlets in *C. daliensis* (C.L.Li) G.Parmar & L.M.Lu. Villous hairs on the branchlets

were observed in *C. mollis* (Wall. ex M.A.Lawson) G.Parmar & J.Wen, *C. fugongensis* (C.L.Li) G.Parmar & L.M.Lu, and *C. ciliifera*. The morphologies of the trichome on branchlets are shown in Figure 2A,B.

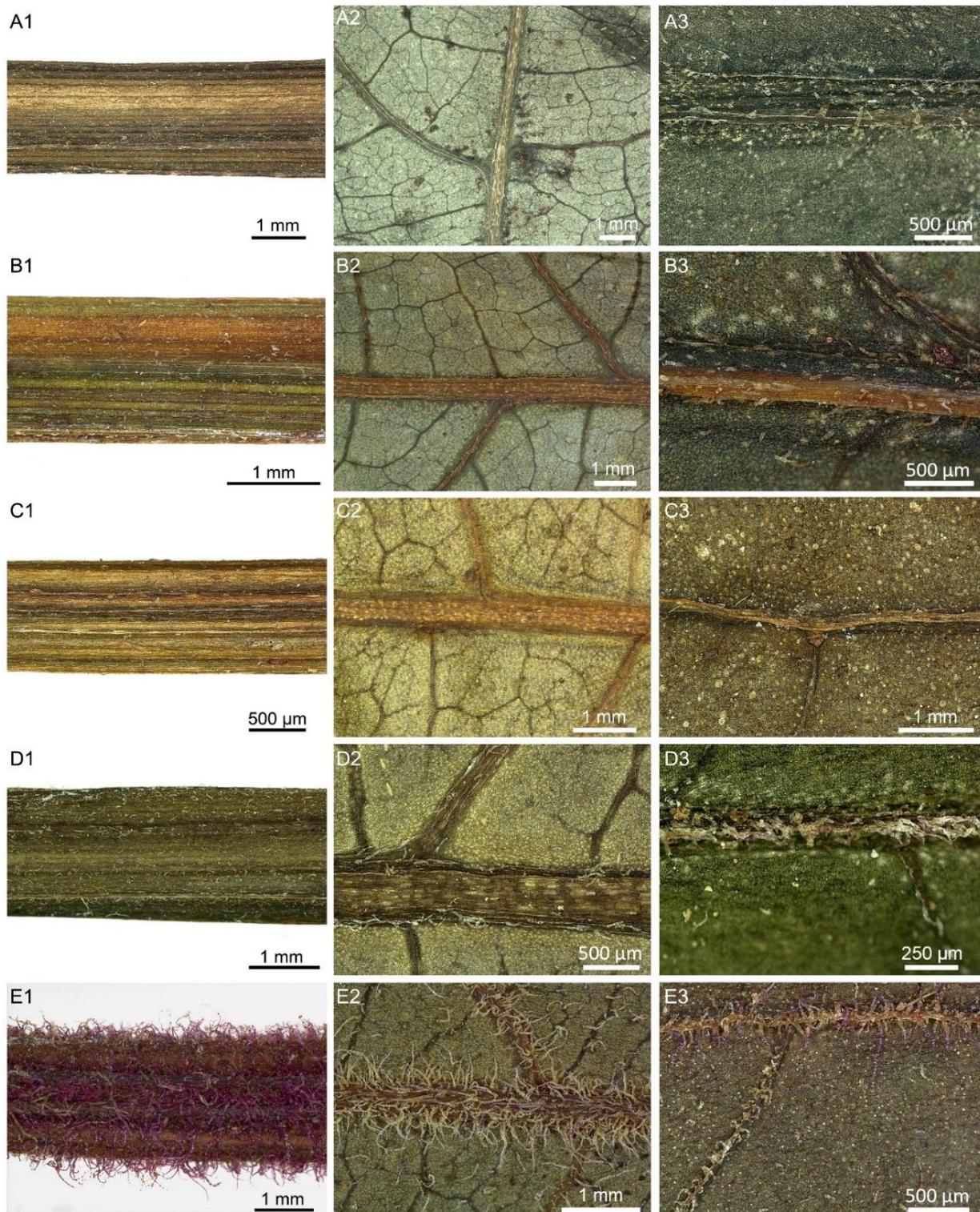


Figure 1. Trichomes' diversity on the midvein in leaves of *Caustonis*. (A) Abaxial leaf surface of *C. timoriensis* var. *mekongensis* with straight hairs (CPG32937). (B) Abaxial leaf surface of *C. fugongensis* with curved hairs. (C) Adaxial leaf surface of *C. trifolia* with hooked hairs (CPG23885). (D) Adaxial leaf surface of *C. fugongensis* with appressed hairs. (E) Abaxial leaf surface of *C. timoriensis* var. *mekongensis* with pilose hairs (CPG18926). (F) Abaxial leaf surface of *C. mollis* with villous hairs.



(A)

Figure 2. Cont.



(B)

Figure 2. (A) Morphological variations of trichomes in *Causonis*. A, *C. clematidea*. B, *C. timoriensis* var. *mekongensis*, CPG32937. C, *C. trifolia*, LA17. D, *C. maritima*. E, *C. fugongensis*. Trichomes' morphologies on branchlets, abaxial, and adaxial surfaces of leaves are shown from left to right, respectively. (B) Morphological variations of trichomes in *Causonis*. A, *C. japonica* var. *japonica*. B, *C. japonica* var. *pseudotrifolia*. C, *C. corniculata*. D, *C. daliensis*. E, *C. mollis*. Trichomes' morphologies on branchlets, abaxial, and adaxial surfaces of leaves are shown from left to right, respectively.

The abaxial leaf surface had villous hairs in *C. mollis*, *C. fugongensis*, and *C. ciliifera*, while *C. corniculata* and *C. maritima* were glabrous on the abaxial leaf surface. The abaxial leaf surface was glabrous to sparsely pubescent in *C. japonica* var. *japonica*, *C. japonica* var. *pseudotrifolia*, and *C. tenuifolia*, but pubescent with straight hairs in *C. clematidea*, *C. daliensis*, and *C. timoriensis* var. *mekongensis*. Pilose hairs were also observed in *C. timoriensis* var. *mekongensis* and sparsely pubescent to densely pubescent with straight hairs in *C. trifolia* on the abaxial leaf surface, while *C. fugongensis* also had curved hairs on the abaxial leaf surface. The trichomes' morphologies on the abaxial leaf surfaces are shown in Figures 1 and 2A,B.

Villous hairs on the adaxial leaf surface were observed in *C. mollis* and *C. ciliifera*, sometimes confined only to the midvein. Glabrous to sparsely pubescent with simple hairs were observed in *C. clematidea*, *C. japonica* var. *japonica*, and *C. japonica* var. *pseudotrifolia* on the adaxial leaf surface, but pubescent with simple hairs were observed in *C. daliensis*, *C. trifolia*, and *C. timoriensis* var. *mekongensis*. Appressed hairs were found in *C. fugongensis* on the adaxial surface and hooked hairs on the midvein of adaxial surface in *C. maritima*. The morphological variation in trichomes used for the diagnosis of species, including trichome morphology on the adaxial leaf surface, is presented in Table 2 (Figures 1 and 2A,B).

Table 2. Morphology of trichomes on branchlets and leaves.

Taxon	Trichomes on Branchlets	Trichomes on Abaxial Leaf Surface	Trichomes on Adaxial Leaf Surface
<i>C. ciliifera</i>	villous	villous	villous
<i>C. clematidea</i>	pubescent when young	pubescent	sparsely pubescent
<i>C. corniculata</i>	glabrous or sometimes sparsely pubescent	usually glabrous	usually glabrous
<i>C. daliensis</i>	puberulent	pubescent	pubescent
<i>C. fugongensis</i>	villous	villous/curved hairs	appressed hairs
<i>C. japonica</i> var. <i>japonica</i>	glabrous or pilose	glabrous to sparsely pubescent	glabrous to sparsely pubescent
<i>C. japonica</i> var. <i>pseudotrifolia</i>	almost glabrous	glabrous to sparsely pubescent	glabrous to sparsely pubescent
<i>C. maritima</i>	usually glabrous	usually glabrous	hooked hairs
<i>C. mollis</i>	villous	villous	villous
<i>C. tenuifolia</i>	usually glabrous	usually glabrous	usually glabrous
<i>C. timoriensis</i> var. <i>mekongensis</i>	sparsely pubescent or pilose	pilose or with simple hairs	pubescent
<i>C. trifolia</i>	sparsely pubescent	pubescent	pubescent

3.2. Quantitative Study

The length of trichomes on branchlets, as well as the abaxial and adaxial leaf surfaces, was measured in different *Causonis* species. *Causonis corniculata* was found to have the shortest trichomes ($52.716 \pm 28.73 \mu\text{m}$) on branchlets, while *C. mollis* has the longest trichomes ($794.5 \pm 54.32 \mu\text{m}$), followed by *C. trifolia* ($516.3 \pm 466.432 \mu\text{m}$). The observed *C. maritima* from Australia did not have any trichomes on the branchlets. On the abaxial leaf surface of *C. corniculata* and *C. maritima*, there were no trichomes. *Causonis daliensis* possessed the shortest trichomes ($171.5 \pm 114.414 \mu\text{m}$) on the abaxial leaf surface. The longest trichomes on the abaxial leaf surface were observed in *C. mollis* ($533 \pm 318.379 \mu\text{m}$), followed by *C. trifolia* ($467.9 \pm 325.638 \mu\text{m}$). On the adaxial leaf surface, the shortest trichomes were observed in *C. daliensis* ($57.316 \pm 78.233 \mu\text{m}$) and the longest in *C. trifolia* ($491 \pm 421.59 \mu\text{m}$), while trichomes were absent in *C. corniculata*. The trichomes' length of different species is presented in Table 3. In addition, categorical features of trichomes on branchlets and leaves are presented in Table 4.

3.3. Principle Component Analysis (PCA)

Quantitative characters were employed for the PCA, and it was discovered that the first PCA variance was 89.275, while the total of the three PCA variances was 100. The eigenvalue, nevertheless, ranged from 0.3124 to 8.3719. (Table 5 and Figure 3). Based on

trichomes on branchlets, abaxial leaf surfaces, and adaxial leaf surfaces, we investigated species variation. Six taxa representing five species (*C. daliensis*, *C. fugongensis*, *C. mollis*, *C. japonica* var. *japonica*, *C. japonica* var. *pseudotrifolia*, and *C. timoriensis* var. *mekongensis*) occurred in PC1, whereas PC2 was represented by four species (*C. clematidea*, *C. corniculata*, *C. maritima*, and *C. trifolia*).

Table 3. Quantitative features of trichomes on branchlets and leaves.

Taxon	Trichomes' Length on Branchlets Min–Max (µm)	Trichomes' Length on Branchlets (µm)	Trichomes' Length on Abaxial Leaf Surface Min–Max (µm)	Trichomes' Length on Abaxial Leaf Surface (µm)	Trichomes Length on Adaxial Leaf Surface Min–Max (µm)	Trichomes' Length on Adaxial Leaf Surface (µm)
<i>C. clematidea</i>	99.9–455.6	258.5 ± 164.93	99.9–499.9	268.1 ± 155.419	74.1–447.62	253.3 ± 148.996
<i>C. corniculata</i>	24.9–99.9	52.716 ± 28.73	Absent	Absent	Absent	Absent
<i>C. daliensis</i>	33.3–250	130 ± 82.235	53.9–307.7	171.5 ± 114.414	46.5–110.5	57.316 ± 78.233
<i>C. fugongensis</i>	66.7–766.7	360.1 ± 292.73	109.1–454.6	261.9 ± 127.304	87.5–212.5	123.1 ± 52.052
<i>C. japonica</i> var. <i>japonica</i>	66.7–177.8	123.3 ± 42.832	142.9–342.9	245.5 ± 97.376	54.6–254.6	141.66 ± 54.422
<i>C. japonica</i> var. <i>pseudotrifolia</i>	33.3–211.1	115.1 ± 67.466	73.7–368.4	212.9 ± 128.562	43.8–225	115.5 ± 72.78
<i>C. maritima</i>	Absent	Absent	Absent	Absent	83.3–266.7	167.23 ± 77.322
<i>C. mollis</i>	499.9–1034.5	794.5 ± 54.32	100–850	533 ± 318.379	94.1–599.9	216 ± 3.011
<i>C. timoriensis</i> var. <i>mekongensis</i>	49.9–233.3	131.9 ± 77.918	62.5–475	234.8 ± 164.124	80–280	171.1 ± 76.1
<i>C. trifolia</i>	54.6–1090.9	516.3 ± 466.432	90–870	467.9 ± 325.638	60–1020	491 ± 421.59

Table 4. Categorical features of trichomes on branchlets and leaves.

Taxon	Trichomes' Length on Branchlets	Trichomes' Length on Abaxial Leaf Surface	Trichomes' Length on Adaxial Leaf Surface
<i>C. clematidea</i>	3	3	3
<i>C. corniculata</i>	1	0	0
<i>C. daliensis</i>	2	3	1
<i>C. fugongensis</i>	2	3	2
<i>C. japonica</i> var. <i>japonica</i>	2	3	2
<i>C. japonica</i> var. <i>pseudotrifolia</i>	2	3	2
<i>C. maritima</i>	0	0	2
<i>C. mollis</i>	6	5	4
<i>C. timoriensis</i> var. <i>mekongensis</i>	2	3	2
<i>C. trifolia</i>	6	5	6

Table 5. PCA of selected species of *Causonis*.

PC	Eigenvalue	% Variance
1	8.37199	89.275
2	0.6934	7.3941
3	0.312386	3.3311

3.4. Ancestral Character State Reconstruction

Using well-resolved chloroplast phylogeny, three morphological character states of trichomes based on their location in the *Causonis* were investigated for state evolution. According to our findings, the trichomes on branchlets in the genus were “glabrous to sparsely pubescent” in their ancestral state and “villous” in their derived state (Figure 4A). Trichomes on the abaxial leaf surface were “glabrous to sparsely pubescent” in their ancestral state, whereas “villous” was a derived state in the genus (See Figure 4B). Trichomes on the adaxial leaf surface were “glabrous to sparsely pubescent” in their ancestral state, and they were “villous” in their derived state in *Causonis* (Figure 4C).

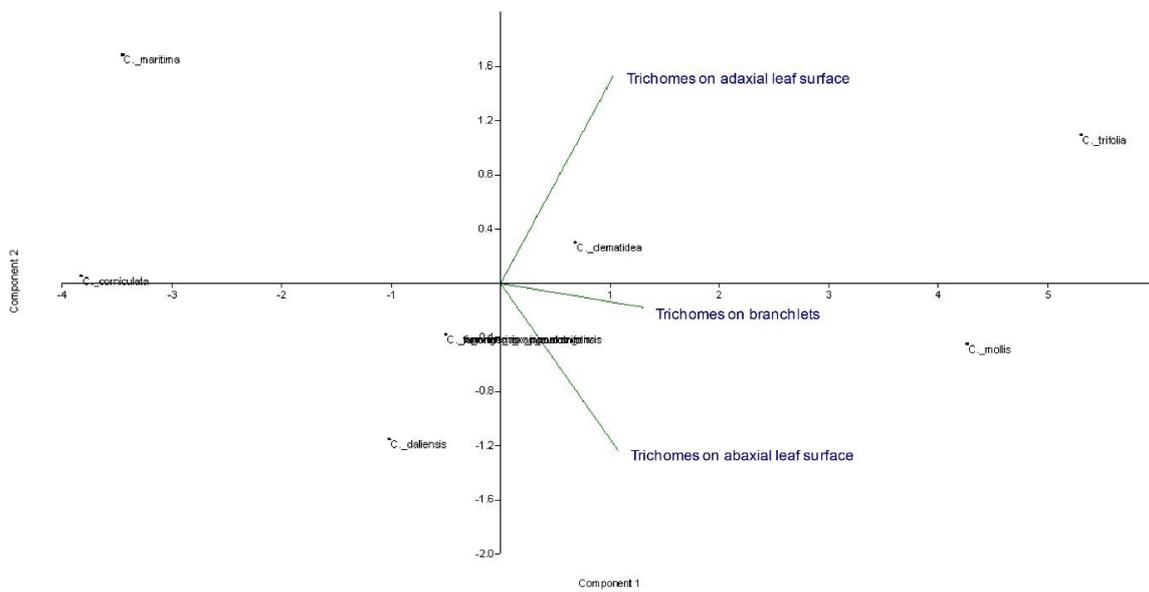


Figure 3. PCA of the selected taxa of *Causernia* based on trichomes on branchlets, abaxial, and adaxial leaf surfaces.

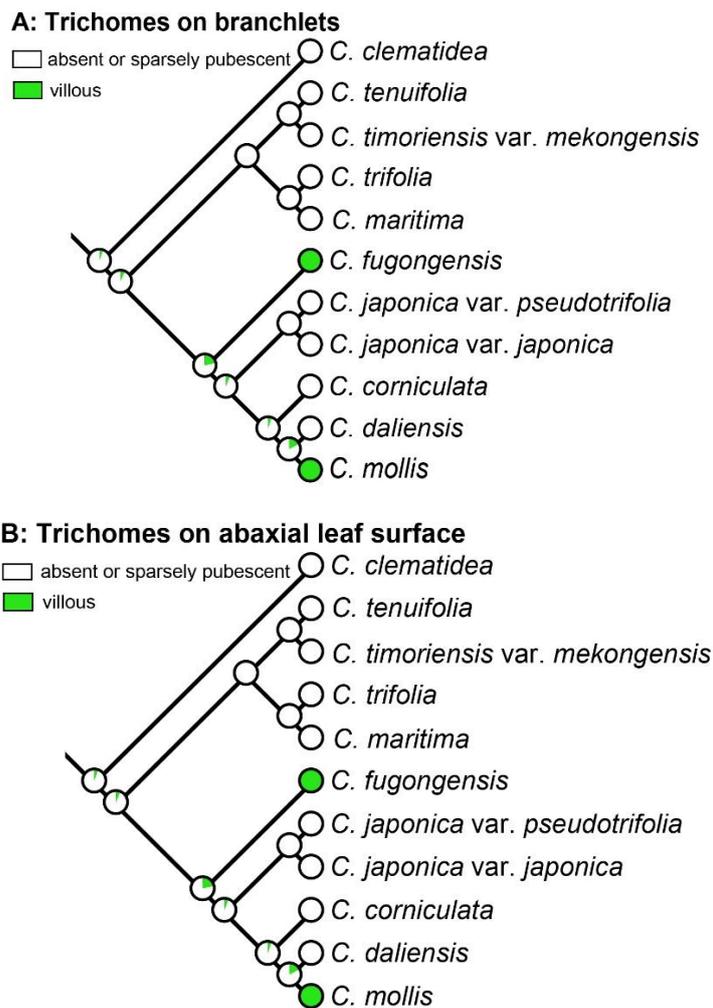


Figure 4. Cont.

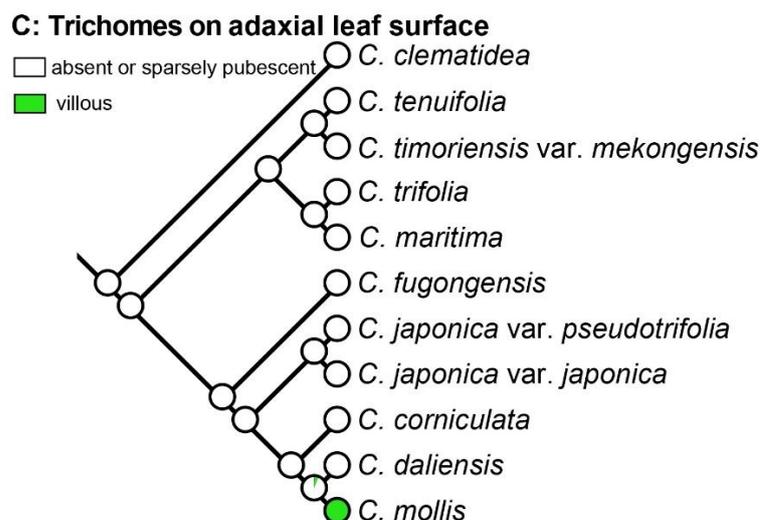


Figure 4. Character optimization of trichomes using the maximum likelihood approach based on the Mk1 model implemented in Mesquite. (A) Trichomes on branchlets. (B) Trichomes on abaxial leaf surface. (C) Trichomes on adaxial leaf surface. Pie charts show each character's ML support at its ancestral nodes.

4. Discussion

The morphology of vegetative parts, such as branchlets and leaves, is found to be highly helpful in identifying Vitaceae taxa [20,21,27,28]. In particular, trichomes on branchlets and leaves are highly helpful for identifying the species of *Causonis* [23,27]. Using field observations and a genus-wide screening of hundreds of herbarium specimens, branchlets were observed to be sparsely pubescent in the young/immature stage, but glabrous in the mature stage in the same plant. Sometimes, densely pubescent hairs were observed in the seedling stage or young stage in some taxa like *C. clematidea* and *C. japonica* var. *japonica*, with the morphology rarely observed in the mature stage. All of the hairs observed in the genus were simple. Glandular or ribbon-like trichomes as revealed in genus *Vitis* by Ma, et al. [28] were not found in *Causonis*. *Causonis*' non-glandular trichomes appear to be sufficient for performing its defensive and protective functions against a variety of stresses, such as serving as a mechanical barrier against low humidity, intense light and high temperatures, and insects' behaviors during oviposition and feeding [30]. Only *C. mollis*, *C. ciliifera*, and *C. fugongensis* were found to have villous hairs on their branchlets. The only species with corniculate petals are *C. ciliifera* and *C. corniculata*, but the branchlets and leaves of *C. ciliifera* are villous, while those of *C. corniculata* are nearly glabrous.

It is very challenging to identify *Causonis* species based solely on trichomes; additional characters such as petals, seeds, stipules, tendrils, or veins, besides trichomes, are needed for the identification. For example, *C. trifolia* and *C. maritima* can be easily distinguished based on trichome morphology, but an additional tendrils' morphology is needed for their confirmation. Leaves of *C. trifolia* are pubescent on both surfaces, while *C. maritima* typically have hooked hairs limited to the midvein on the adaxial surface and have a glabrous abaxial surface [27,31]. However, *C. trifolia* occasionally exhibits hooked hairs on the adaxial surface. Similarly, the abaxial leaf surface has villous hairs that limit the number of species to three: *C. ciliifera*, *C. mollis*, and *C. fugongensis*, for easy identification in the genus. *Causonis ciliifera* can be easily differentiated from the other two as having corniculate petals, considering an additional character. The appressed hairs on the adaxial leaf surface or the prominently raised veins on the abaxial leaf surface are characteristics that help identify *C. fugongensis*. On the abaxial leaf surface of *C. fugongensis*, villous hairs or curved hairs have been observed. *Causonis mollis* requires the aid of seed characters like ventral infolds shape or endosperm shape in the cross section of seed for its identification.

Causonis corniculata can be differentiated from *C. ciliifera*, the only another species with corniculate petals in the genus, as lacking villous hairs on branchlets and leaves, as the former generally has glabrous branchlets and leaves. *Causonis tenuifolia* and *C. corniculata* are both almost glabrous, but the former lacks corniculate petals, and the petals' morphology here is another additional character that helps in identifying both species. *Causonis timoriensis* var. *mekongensis* can be distinguished from other trifoliolate species like *C. trifolia*, *C. maritima*, and *C. japonica* var. *pseudotrifolia* by its pilose hairs on the abaxial leaf surface [20]. Without taking into account the morphological variation in trichomes, no other characters are sufficient to distinguish those species. As a result, trichomes' morphology plays a very useful role in species identification in the genus.

High variations were observed in the length of trichomes between the species. However, the length of trichomes in a species revealed a certain degree of uniformity. For example, *C. corniculata*, which lacks trichomes on the abaxial and adaxial leaf surfaces, has the shortest trichomes on branchlets, and *C. mollis* and *C. trifolia* both share the longest trichomes on all of the studied three surfaces between them. Therefore, the length of trichomes revealed a certain degree of inference in the genus.

This study showed that the glabrous to slightly pubescent trichomes are the ancestral state and the villous hairs are the derived state in the genus *Causonis*, regardless of the position of the trichomes in the genus, such as branchlets or leaves (abaxial and adaxial). It is possible that the villous hairs on branchlets and the abaxial surface of leaves have evolved at least twice, while they have evolved once on the adaxial leaf surface in the genus. This suggests that branchlets that are glabrous, sparsely pubescent, pubescent, or puberulent with normal hairs and sometimes pilose are the ancestral state of the genus. Glabrous, sparsely pubescent, or pubescent with normal hairs and sometimes pilose, hooked, curved, or appressed hairs on the leaflets' surface (abaxial and adaxial) are also the ancestral state of the genus.

5. Conclusions

The current study is the first comprehensive examination of the micromorphology of the trichomes in *Causonis*. Twelve taxa were studied for the morphology of trichomes on branchlets and abaxial and adaxial surfaces of leaves using a stereomicroscope. The genus was found to be represented by only simple trichomes that are non-glandular and unbranched. Different types of trichomes such as straight, curved, hooked, appressed, and villous are found in branchlets and leaves in the *Causonis*. High variations are noticed in the trichomes' length between the species. Based on the characteristics of the micromorphological trichomes, PCA is used to determine the species boundary of the taxa. Besides, we examined the character state evolution of trichomes on leaf surfaces, including the abaxial and adaxial leaf surfaces based on the chloroplast dataset using the maximum likelihood approach in Mesquite. Glabrous to sparsely pubescent hairs, irrespective of the position in plants, are the ancestral state and villous hairs are the derived state in the genus. Trichomes' morphology is found to be very useful in the identification of *Causonis* species. To uncover the whole trichome diversity within the family, a similar study should be conducted on additional genera of the Vitaceae.

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