



Morphological studies on leaf epidermis in six species of *Nekemias* Raf. (Vitaceae) from China

ZHAO-FU CHU^{1,3}, RAN MENG^{2,4}, YING MENG^{2,5}, KUN SONG^{1,6}, LIANG-JUN DA^{1,7*} & ZE-LONG NIE^{2,8*}

¹Tiantong National Forest Ecosystem Observation and Research Station, Shanghai Key Lab for Urban Ecological Processes and Eco-Restoration, School of Ecological and Environmental Sciences, East China Normal University, Dongchuan Road 500, Shanghai 200241, China

²College of Biology and Environmental Sciences, Jishou University, Jishou, Hunan 416000, China

³ Email? Orcid ID?

⁴ ?

⁵ ?

⁶ ljda@xauat.edu.cn; Orcid ID?

⁷ niez@jsu.edu.cn; Orcid ID?

*Authors for correspondence: ljda@xauat.edu.cn; niez@jsu.edu.cn

Abstract

Nekemias Raf. (Vitaceae) is a re-defined genus isolated from the traditional *Ampelopsis* in the grape family of Vitaceae, including eight species mainly distributed in eastern Asia, and one in North America. In this study, the leaf epidermis of 28 samples representing six species of *Nekemias* and one species from the related genus of *Ampelopsis* are investigated using light microscopy. The results show that the shapes of leaf epidermal cells are polygonal or irregular. The variations of anticlinal walls are found commonly among species of *Nekemia*, especially within *N. cantoniensis* and *N. grossedentata*, which is probably associated with their habitats. Irregular stomata are found on the abaxial surface. The epidermal hair styles are multicellular, single-row on both sides and common in *N. cantoniensis*, *N. grossedentata*, and *N. rubifolia*, not in *N. chaffanjonii* and *N. hypoglauca*. Our results highlight that the variation of leaf epidermis in *Nekemias* are possibly related to the environment and habitats.

Keywords: anticlinal walls, environmental adaptation, interspecific difference, diversity

Introduction

Nekemias Raf. (1838:87), as a member of the basal lineage of tribe Ampelopsidae in Vitaceae (Wen *et al.*, 2018), is characterized by the pinnately to bipinnately compound leaves, complex axillary buds, and more floriferous than the closely related *Ampelopsis* Michaux (1803:159) (Galet, 1967; Wen *et al.*, 2014). Members of the genus had been placed in *Ampelopsis* sect. *Leeaceifoliae*, which was recently raised into the generic level based on both morphological and phylogenetic evidence (Ren *et al.*, 2011; Soejima & Wen, 2006; Wen *et al.*, 2014; Wen *et al.*, 2007; Zhang *et al.*, 2015).

To date, there are nine species belonging to the genus, which shows a disjunctive distribution between North America and eastern Asia (Nie *et al.*, 2012; Wen, 1999). However, only one species, *Nekemias arborea* (L.) J. Wen & Boggan (2014:13), occurs in North America. The eastern Asian members of *Nekemias* are widespread from tropical and subtropical to temperate regions (Chen *et al.*, 2007; Wen *et al.*, 2014). Phylogenetic analyses supported the North American species sister to a clade including all from the eastern Asia (Nie *et al.*, 2012). Two lineages were recognized within the eastern Asian group, with *N. megalophylla* (Diels & Gilg) J. Wen & Z.L. Nie (2014:16) and *N. hypoglauca* (Hance) J. Wen & Z.L. Nie (2014:16) forming a clade sister to the remainder (Nie *et al.*, 2012). However, morphological evolution in the *Nekemias*, especially for the eastern Asian taxa, is complex and there are very few morphological characters supporting these two lineages (Wen, 2007; Chen & Manchester, 2011). Furthermore, taxonomic classification and species identification within the genus mainly rely on vegetative characters, such as leaf morphology.

Foliar epidermis is one of the most widely considered of all the non-reproductive traits and has been widely used

to assist morphological delimitation. This is well documented in botanical literature for infrageneric classification and species circumscription (Stace, 1965; Metcalfe & Chalk, 1988; Klich, 2000; Chen *et al.*, 2009; Ullah *et al.*, 2018). Increasing attention is paid to leaf epidermal characters aimed at investigating whether the microcharacters are more conservative and better taxonomic indicators than the macrocharacters in the delimitation and classification of plants (Meng *et al.*, 2016; Nurul-Aini *et al.*, 2014; Stace, 1984). Differences in epidermal characters between and within species may be caused by their genetic variation (Liao *et al.*, 2008), changeable habitat environment (Klich, 2000; Falcão *et al.*, 2015), or mostly as a result of both them (Nitta & Ohsawa, 1997).

Ren *et al.* (2003) has investigated 34 species representing eleven genera of Vitaceae and suggested that the microcharacters in the leaf epidermis could provide good evidence for intergeneric classification and species circumscription. But no studies have investigated on epidermal characters of *Nekemias*. In the present study, foliar epidermis features of six species of *Nekemias* are examined and described using light microscopy. Additionally, multiple individuals are sampled per species with a wide distribution range, and differences between species are compared and their possible systematic implications are discussed in relation to the molecular phylogeny. Therefore, the aim of this article is to use foliar epidermis characters for species identification and taxonomy to form a reliable classification that corresponds to recent phylogenetic results inferred for *Nekemias*.

Materials and methods

Mature leaves were taken from 28 herbarium specimens representing all six species of *Nekemias* from China, except for the endemic species *N. gongshanensis* (Hance) J. Wen & Z.L. Nie (2014:15). We also included *Ampelopsis delavayana* Planch. ex Franch. (1886: 460) as its sister genus. Moreover, multiple individuals throughout the geographic distribution including subtropical and temperate areas of China have been sampled for the widely distributed species, such as *N. cantoniensis* (Hook. & Arn.) J. Wen & Z.L. Nie (2014:13), and *N. grossedentata* (Hand. -Mazz.) J. Wen & Z.L. Nie (2014:16) (Table 1).

Leaf materials were cut from the mid-lamina region and macerated in 1% Jeffrey's solution (equal volumes of 10% aqueous CrO₃ and 10% HNO₃) to obtain the epidermis tissues. Following the treatments of Meng *et al.* (2016), leaf epidermis tissue was stained in a solution of 1% safranin-alcohol (50%), and then dehydrated in an ethanol series before being mounted in gum. To check the consistency of epidermal features, at least two leaves were collected for each individual, and more than five slides were made from different parts of a single leaf for each taxon. Descriptive terminology for leaf epidermia characters follows Fryns-Claessens & Cotthem (1973), Wilkinson (1979), and Baranova (1992).

We also mapped the character states of anticlinal wall types and corresponding habitats to a maximum likelihood tree, which was obtained from Chu (2019) with taxa pruned to represent the corresponding taxa with the same vouchers investigated in this study.

Results

All leaf anatomy characters (e.g., epidermal hairs, types of anticlinal wall and stomatal characteristics) of 28 samples are shown and illustrated in Table 1 and Figures 1, 2. More than three samples of foliar characteristics were observed for each sample.

A total of 11 individuals were sampled from different areas throughout distribution areas of *N. grossedentata*. All of them share a sinuolate pattern of anticlinal wall on both sides of leaves (Figures 1, 2A–D, F–G, I–K), except for one population from Fujian showing sinuate anticlinal walls on the abaxial side and straight on the adaxial side (Figures 1, 2E), and one from Guangdong with sinuate anticlinal walls on both surfaces of leaves (Figures 1, 2H).

Seven samples from different areas throughout the distribution range of *N. cantoniensis* were observed with their abaxial anticlinal walls divided into three types (Table 1). Four of them from Guangxi, Guangdong and Fujian are shown with straight types (Figures 1, 2L–M, O–P), two from Jiangxi (Figure 1Q) and Guizhou (Figure 1R) appear as arched type, and one from Hainan shown as sinuolate type (Figure 1N). The adaxial anticlinal walls also were recognized into three types. Four of which were collected from Guangxi (two), Hainan, and Jiangxi are shown as sinuolate type (Figure 2L–N, Q), with two from Guangdong and Fujian as straight type (Figure 2O–P) and one with arched type from Guizhou (Figure 2Q).

TABLE 1. Taxa, localities, voucher specimens and characteristics of leaf epidermis of *Nekemias* and *Ampelopsis*.

| Taxon | Voucher | Geographic information | Locality | Habitat | Abaxial anticlinal wall | Adaxial anticlinal wall | Stoma | Figures |
|-------------------------------|------------|---|--------------------|---------------------|-------------------------|-------------------------|-----------|------------|
| | Chu 001 | N25° 05' 18.21"; E109° 08' 39.91"; 515 m | Rongshui, Guangxi | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2A |
| | Chu 007 | N22° 53' 11.37"; E108° 13' 59.81"; 950 m | Wuming, Guangxi | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2B |
| | Chu 017 | N25° 11' 52.17"; E113° 13' 59.81"; 265 m | Lechang, Guangdong | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2C |
| | Chu 037 | N23° 00' 38.81"; E111° 13' 59.81"; 122 m | Yunfu, Guangdong | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2D |
| | Chu 049 | N21° 49' 34.71"; E111° 16' 16.82"; 545 m | Dianbai, Guangdong | Forest edge, sunny | sinuate | sinuate | irregular | Figs 1,2E |
| <i>Nekemias grossedentata</i> | Chu 051 | N21° 49' 31.05"; E111° 16' 14.00"; 330 m | Dianbai, Guangdong | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2F |
| | Chu 074 | N27° 38' 15.51"; E117° 55' 13.56"; 750 m | Wuyishan, Fujian | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2G |
| | Chu 086 | N28° 07' 35.10"; E118° 34' 50.33"; 122 m | Pucheng, Fujian | Under forest, shady | straight | sinuate | irregular | Figs 1,2H |
| | Chu 205 | N25° 17' 25.68"; E115° 25' 51.99"; 1350 m | Anyuan, Jiangxi | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2I |
| | Chu 226 | N25° 23' 31.13"; E114° 04' 49.15"; 756 m | Dayu, Jiangxi | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2J |
| | Chu 247 | N28° 25' 45.18"; E108° 55' 28.05"; 853 m | Xiushan, Chongqing | Forest edge, sunny | sinuolate | sinuolate | irregular | Figs 1,2K |
| | Chu 002 | N24° 21' 33.82"; E106° 33' 49.03"; 439 m | Baise, Guangxi | Under forest, shady | straight | sinuolate | irregular | Figs 1,2L |
| | Chu 011 | N22° 53' 14.37"; E108° 13' 60.00"; 422 m | Wuming, Guangxi | Under forest, shady | straight | sinuolate | irregular | Figs 1,2M |
| <i>N. cantoniensis</i> | Chu 015 | N19° 29' 48.03"; E109° 24' 0.93"; 232 m | Danzhou, Hainan | Forest edge, sunny | sinuolate | straight | irregular | Figs 1,2N |
| | Chu 022 | N25° 11' 53.17"; E113° 13' 59.00"; 395 m | Lechang, Guangdong | Forest edge, shady | straight | straight | irregular | Figs 1,2O |
| | Chu 061 | N26° 47' 23.52"; E116° 53' 12.65"; 696 m | Jianning, Fujian | Forest edge, shady | straight | straight | irregular | Figs 1,2P |
| | Chu 218 | N25° 23' 31.13"; E114° 04' 49.15"; 350 m | Dayu, Jiangxi | Under forest, shady | arched | arched | irregular | Figs 1,2Q |
| | Chu 235 | N27° 54' 40.26"; E108° 41' 37.34"; 900 m | Tongren, Guizhou | Forest edge, shady | arched | arched | irregular | Figs 1,2R |
| <i>N. chatfanjoni</i> | Nie 4536 | N29° 12' 42.53"; E109° 25' 25.35"; 854 m | Longshan, Hunan | Forest edge, shady | arched | arched | irregular | Figs 1,2S |
| | Nie 4544 | N29° 56' 15.95"; E110° 46' 42.73"; 950 m | Shimen, Hunan | Forest edge, shady | arched | arched | irregular | Figs 1,2T |
| | Chu 025 | N25° 11' 55.17"; E113° 13' 57.00"; 875 m | Lechang, Guangdong | Forest edge, shady | arched | arched | irregular | Figs 1,2U |
| <i>N. rubifolia</i> | Chu 238 | N27° 54' 44.26"; E108° 41' 38.34"; 934 m | Tongren, Guizhou | Forest edge, shady | arched | arched | irregular | Figs 1,2V |
| | Chu 239 | N27° 54' 48.26"; E108° 41' 38.00"; 900 m | Tongren, Guizhou | Forest edge, shady | arched | arched | irregular | Figs 1,2W |
| <i>N. megalophylla</i> | ZDG 201706 | N30° 04' 43.37"; E110° 33' 6.59"; 1350 m | Wufeng, Hubei | Forest edge, shady | arched | sinuolate | irregular | Figs 1,2X |
| | Chu 401 | N31° 29' 6.08"; E109° 58' 17.49"; 1200 m | Shenmongjia, Hubei | Forest edge, shady | arched | sinuolate | irregular | Figs 1,2Y |
| | Chu 206 | N25° 17' 25.68"; E115° 25' 51.99"; 564 m | Anyuan, Jiangxi | Forest edge, shady | arched | arched | irregular | Figs 1,2Z |
| <i>N. hypoglauca</i> | Chu 207 | N25° 17' 11.37"; E115° 18' 55.81"; 753 m | Anyuan, Jiangxi | Forest edge, shady | arched | arched | irregular | Figs 1,2Aa |
| <i>Ampelopsis delavayana</i> | Nie 4438 | N29° 12' 42.55"; E109° 25' 25.35"; 863 m | Longshan, Hunan | Forest edge, sunny | sinuolate | sinuate | irregular | Figs 1,2Ab |

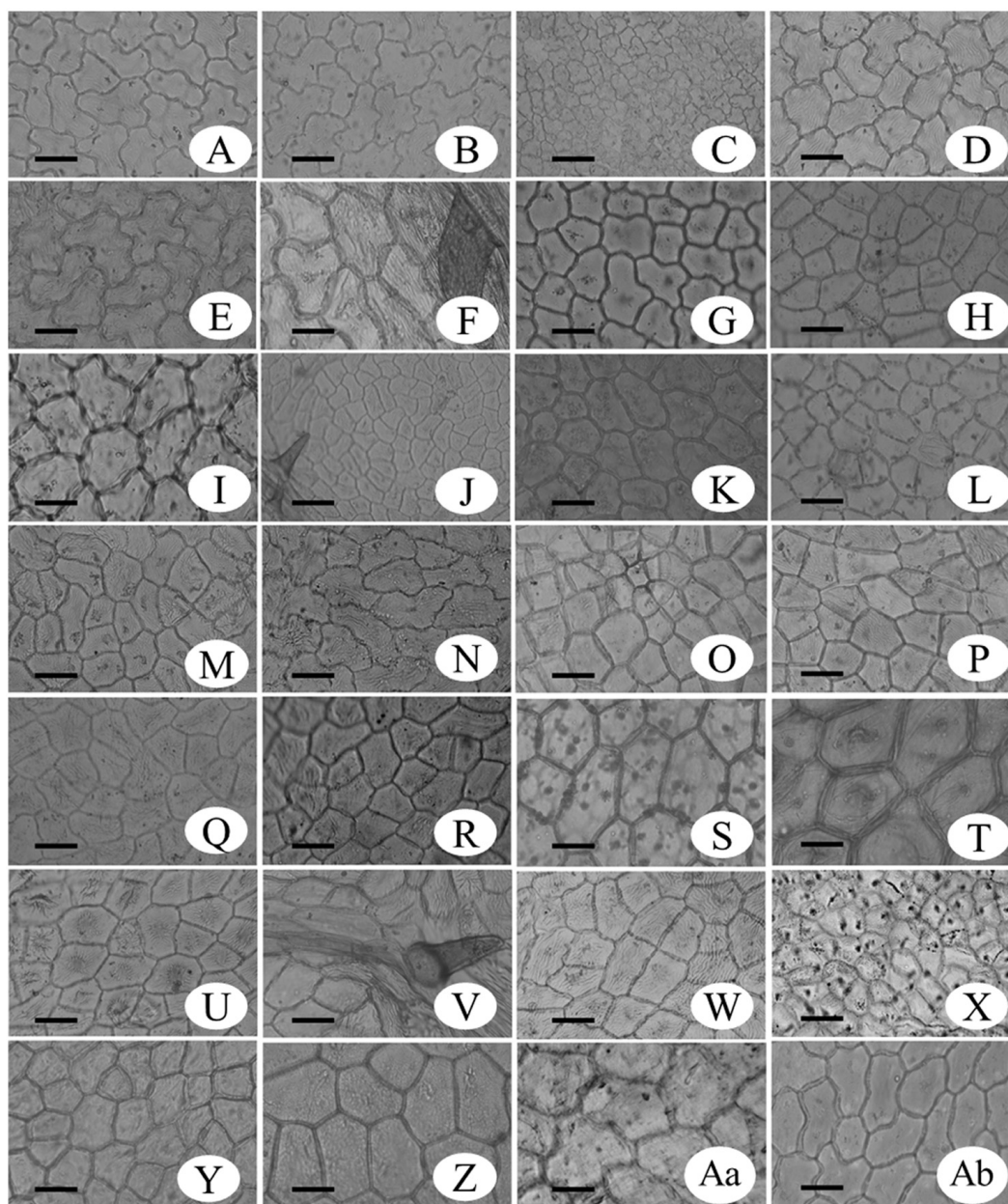


FIGURE 1. Micrographs of the adaxial leaf of *Nekemias* and *Ampelopsis* species.

A. *N. grossedentata* (Chu 001); **B.** *N. grossedentata* (Chu 007); **C.** *N. grossedentata* (Chu 017); **D.** *N. grossedentata* (Chu 037); **E.** *N. grossedentata* (Chu 049); **F.** *N. grossedentata* (Chu 051); **G.** *N. grossedentata* (Chu 074); **H.** *N. grossedentata* (Chu 086); **I.** *N. grossedentata* (Chu 205); **J.** *N. grossedentata* (Chu 226); **K.** *N. grossedentata* (Chu 247); **L.** *N. cantoniensis* (Chu 002); **M.** *N. cantoniensis* (Chu 011); **N.** *N. cantoniensis* (Chu 015); **O.** *N. cantoniensis* (Chu 022); **P.** *N. cantoniensis* (Chu 061); **Q.** *N. cantoniensis* (Chu 218); **R.** *N. cantoniensis* (Chu 235); **S.** *N. chaffanjonii* (Nie 4536); **T.** *N. chaffanjonii* (Nie 4544); **U.** *N. rubifolia* (Chu 025); **V.** *N. rubifolia* (Chu 238); **W.** *N. rubifolia* (Chu 239); **X.** *N. megalophylla* (ZDG 201706); **Y.** *N. megalophylla* (Chu 401); **Z.** *N. hypoglauca* (Chu 206); **Aa.** *N. hypoglauca* (Chu 207); **Ab.** *Ampelopsis delavayana* (Nie 4438). Scale bar = 50µm.

The other four species (i.e., *N. chaffanjonii* (H. Lév. & Van.) J. Wen & Z.L. Nie (2014:14), *N. megalophylla*, *N. hypoglauca*, and *N. rubifolia* (Wall.) J. Wen & Z.L. Nie (2014:14)) have the arched type in the anticlinal walls on both sides (Figures 1S–Aa, 2S–W, Z–Aa) except *N. megalophylla* with sinuolate type on adaxial side (Figure 2X–Y). *Ampelopsis delavayana* exhibits the sinuolate type on the abaxial side and the sinuate type on the adaxial side (Figures 1, 2Ab).

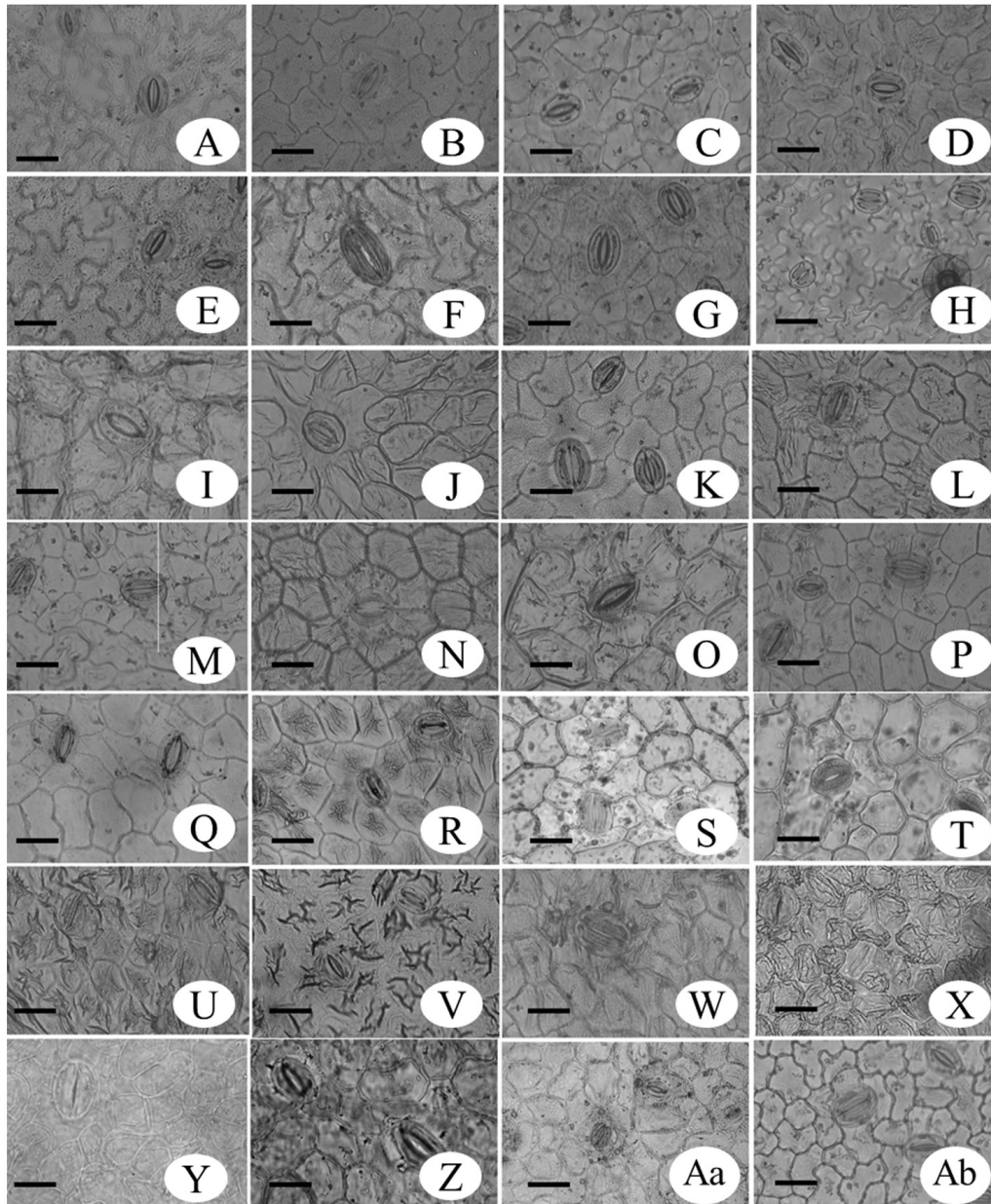


FIGURE 2. Micrographs of the abaxial leaf of *Nekemias* and *Ampelopsis* species.

A. *N. grossedentata* (Chu 001); **B.** *N. grossedentata* (Chu 007); **C.** *N. grossedentata* (Chu 017); **D.** *N. grossedentata* (Chu 037); **E.** *N. grossedentata* (Chu 049); **F.** *N. grossedentata* (Chu 051); **G.** *N. grossedentata* (Chu 074); **H.** *N. grossedentata* (Chu 086); **I.** *N. grossedentata* (Chu 205); **J.** *N. grossedentata* (Chu 226); **K.** *N. grossedentata* (Chu 247); **L.** *N. cantoniensis* (Chu 002); **M.** *N. cantoniensis* (Chu 011); **N.** *N. cantoniensis* (Chu 015); **O.** *N. cantoniensis* (Chu 022); **P.** *N. cantoniensis* (Chu 061); **Q.** *N. cantoniensis* (Chu 218); **R.** *N. cantoniensis* (Chu 235); **S.** *N. chaffanjonii* (Nie 4536); **T.** *N. chaffanjonii* (Nie 4544); **U.** *N. rubifolia* (Chu 025); **V.** *N. rubifolia* (Chu 238); **W.** *N. rubifolia* (Chu 239); **X.** *N. megalophylla* (ZDG 201706); **Y.** *N. megalophylla* (Chu 401); **Z.** *N. hypoglauca* (Chu 206); **Aa.** *N. hypoglauca* (Chu 207); **Ab.** *Ampelopsis delavayana* (Nie 4438). Scale bar = 50µm.

The irregular pattern of the stomata is found for all the samples on the adaxial surfaces within *Nekemias* (Table 1). The structure of the foliar surface hairs is categorized into three types in *Nekemias*. The glabrous foliar surface on both sides is shown with *N. hypoglauca* and *N. chaffanjonii* (Figure 3A, B). The second foliar surface type is sparsely pubescent primarily on veins with cone-shaped, apex blunt and papillate hairs in *N. cantoniensis* (Figure 3C), *N. grossedentata* (Figure 3D), and *N. megalophylla* (Figure 3E). *Ampelopsis delavayana* also has this type of hair (Figure 3F). The third foliar surface type is only found in *N. rubifolia*, with densely gracile villous hairs only present on the adaxial surface (Figure 3G, H).

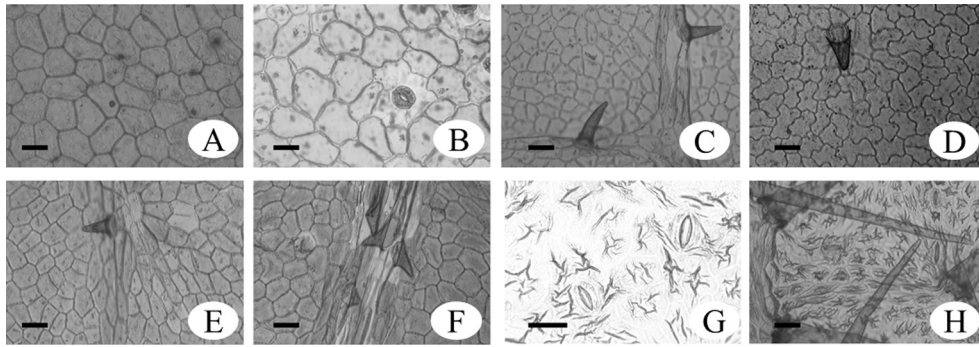


FIGURE 3. The hair types of *Nekemias* and its related species of *Ampelopsis*.

A. *N. hypoglauca* (Chu 206); B. *N. chaffanjonii* (Nie 4544). C–F: The venations and epidermal cells with sparsely pubescent, C. *N. cantoniensis* (Chu 007), D. *N. grossedentata* (Chu 001); E. *N. megalophylla* (Chu 401), F. *Ampelopsis delavayana* (Nie 4438), G–H: the epidermal cells with bending villous. G. *N. rubifolia* (Chu 238), H. *N. rubifolia* (Chu 025). Scale bar = 50µm.

Variations of anticlinal wall are observed and mapped to the maximum likelihood tree to show the correlation between the leaf epidermis characters and phylogenetic relationships (Figure 4). The sunny or shady habitat of each taxon was also mapped on the tree (Figure 4). We found that the collections growing on the sunny areas of the forest edges usually have the sinuate or sinuolate types of anticlinal walls, while those from the shady under forest commonly have the straight to arched anticlinal walls (Figure 4).

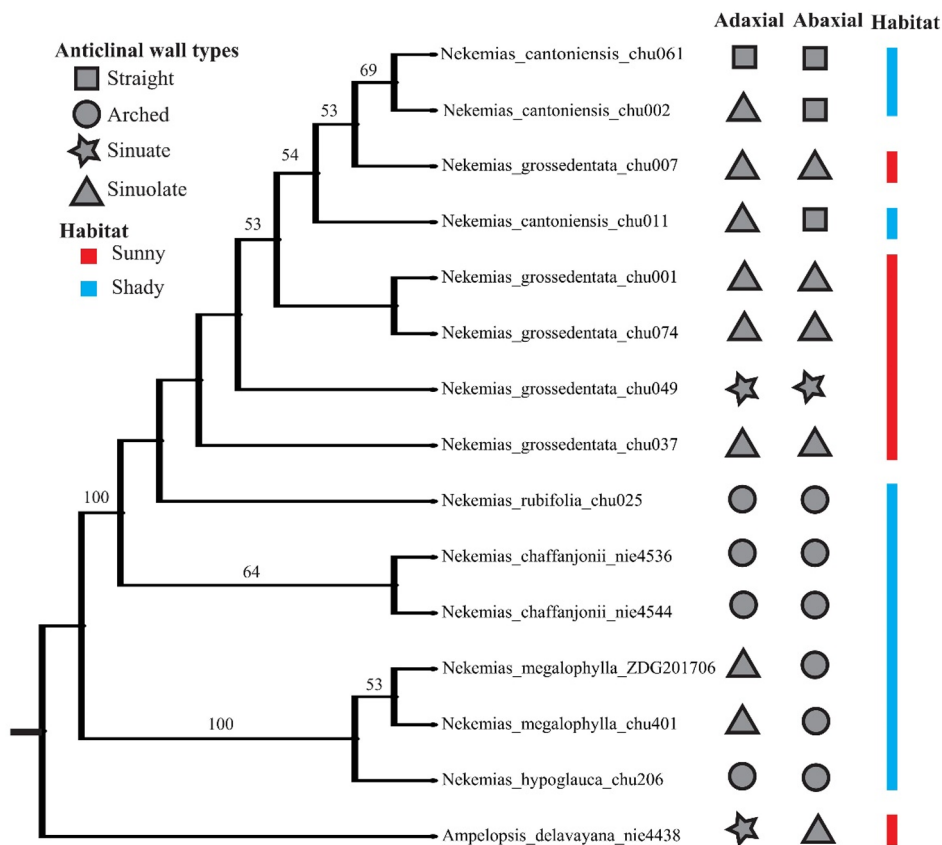


FIGURE 4. A maximum likelihood tree showing the types of anticlinal wall of both leaf sides and habitats.

Discussion

Large variations of anticlinal wall are observed within the widespread species of *N. cantoniensis* and *N. grossedentata*, which may be in connection with their distributions and habitats (Table 1, Figure 4). For instance, samples of *N.*

cantoniensis sampled from tropical regions have the straight type on both sides (Figures 1, 2O–P), while subtropical samples have the sinuolate type and those from temperate have the arched type (Table 1). Although the nine populations of *N. grossedentata* show high consistency in having the sinuolate type among different areas (Figures 1, 2A–D, F–G, I–K), they commonly grow in sunny slopes near the forest and with poor soil. Only three populations growing in shady habitats, show varying degrees of variation on the types of the anticlinal wall (Figures 1, 2E,H). Therefore, the differences of anticlinal wall from different areas within *Nekemias*, especially in *N. grossedentata* and *N. cantoniensis*, suggest that the intraspecific differences may be caused by the climatic and habitat differences where the species are living (Table 1, Figure 4). Moreover, chemical compositions of *N. grossedentata* vary greatly from different distribution areas (Xia, 2016).

Variations of anticlinal wall have been reported in many angiosperm plants, which may be coupled with several environment factors, such as elevation, latitude, and also with the temperature, water, and sunshine (Al-Edhari *et al.*, 2017; Fontenelle *et al.*, 2008; Kaya & Koca, 2003; Meng *et al.*, 2016; Neto *et al.*, 2017). Similar variation patterns of the anticlinal wall within *N. grossedentata* and *N. cantoniensis* are also observed in other plants, such as *Maianthemum* Wiggers (1780:14) (Meng *et al.*, 2016), *Eugenia* Linnaeus(1753:616) (Fontenelle *et al.*, 2008), and *Kalanchoë pumila* Baker(1883:139) (Chernetskyy & Weryszko-Chmielewska, 2008).

The polygonal epidermis and irregular stomata cells, with straight-arch, sinuolate (rarely sinuate) anticlinal wall types in *Nekemias* species (Table 1) are also consistent with previous findings in Vitaceae (Ren *et al.*, 2003). The similar microstructures of the leaf epidermis indicate a close relationship between *Nekemias* and other genera of tribe Ampelopsidae (e.g. *Ampelopsis*, *Clematicissus*, *Rhoicissus*) and tribes Parthenocisseae and Viteae in Vitaceae (Ren *et al.*, 2003; Wen *et al.*, 2018). For example, Lu *et al.* (2012) reported that seven *Parthenocissus* species also have straight-arch, sinuolate anticlinal wall types, with polygonal or irregular epidermis cells. All these genera usually have 5-merous flowers, mainly distributed in temperate regions (Trias-Blasi *et al.*, 2012; Ren *et al.*, 2011; Soejima & Wen, 2006; Wen *et al.*, 2018; Wen *et al.*, 2007; Wen *et al.*, 2013; Zhang *et al.*, 2015). The other tribes of Vitaceae (e.g., Cisseae and Cayratieae) have 4-merous flowers distributed mainly in tropical regions, and normally have irregular epidermis, various patterns of stomata cells (e.g., the hemiparacytic, cyclocytic and staurocytic pattern), and a sinuolate anticlinal wall (Ren *et al.*, 2003).

Hair is an important appendage on the leaf epidermis, which not only increases the thickness of leaf epidermis and decreases transpiration rate, but also defenses from insects, frost, UV-B radiation and mechanical damage (Ishida *et al.*, 2008; Johnson, 1975; Serna & Martin, 2006; Yan *et al.*, 2012). The tropical species (e.g., *N. grossedentata* and *N. cantoniensis*) have sparsely pubescent primarily on veins with cone-shaped, apex blunt and papillate hairs, but the temperate species have densely gracile villous hairs only present on the adaxial surface (e.g., *N. rubifolia*) or glabrous (e.g., *N. hypoglauca* and *N. chaffanjonii*) (Table 1 and Figure 3). *Ampelopsis* has a sparsely pubescent leaf surface (e.g., *Ampelopsis delavayana*), and all three types of hairs are found in *Nekemias* (Figure 3). Therefore, it seems that the stabilizing effect of intraspecific characteristics of hair variation show a close relationship to the tropical and temperate habitats among *Nekemias* species.

Conclusion

In the present study, we demonstrated variations of the leaf anatomy of six *Nekemias* species from China with different types of anticlinal walls, epidermal hair structures, and irregular stomata. Particularly, multiple accessions from two widespread species of *N. grossedentata* and *N. cantoniensis* show a high level of intraspecific variations of the anticlinal wall. However, the rest four *Nekemias* species show consistency of the anticlinal walls on both sides, which may be caused by the limited collections to represent their diversity. Although not all variable characters yield systematic insights, some of them (e.g., anticlinal wall) are possibly related to environment and habitats. Future work with multiple samples from each species to cover the complete distribution are needed to test if the relationships of leaf epidermis traits are related to environmental or genetic factors.

Acknowledgements

This work was supported by the grant from the Natural Science Foundation of China (32060055). We thank Prof. Jun

Wen from the National Museum of Natural History of the Smithsonian Institution for species identification, Mr. Daigui Zhang from Jishou University for kindly helping with sample collection and Mr. Minghe Wu from Jishou University for assistance in the laboratory work.

References

- Al-Edhari, A.H., Sardar, A.S. & Regawi, S.M.A. (2017) A Comparative Anatomical Study for the species of the genus *Carex* L. (Cyperaceae) in Iraq. *Journal of Scientific and Engineering Research*, 4(10), 374–379.
<https://doi.org/10.1600/036364412X656437>
- Baker, J. G. (1883) Contributions to the Flora of Madagascar.—Part I. Polypetalæ. *Journal of the Linnean Society of London, Botany*, 20(126), 87–158.
<https://doi.org/10.1111/j.1095-8339.1883.tb00195.x>
- Baranova, M. (1992) Principles of comparative stomatographic studies of flowering plants. *Botanical Review*, 58(1), 49–99.
<https://doi.org/10.1007/BF02858543>
- Chen, G., Sun, W.B. & Sun, H. (2009) Morphological characteristics of leaf epidermis and size variation of leaf, flower and fruit in different ploidy levels in *Buddleja macrostachya* (Buddlejaceae). *Journal of Systematics and Evolution*, 47(3), 231–236.
<https://doi.org/10.1111/j.1759-6831.2009.00026.x>
- Chen, I. & Manchester, S.R. (2011) Seed morphology of Vitaceae. *International Journal of Plant Sciences*, 172(1), 1–35.
<https://doi.org/10.1086/657283>
- Chen, Z.D., Ren, H. & Wen, J. (2007) *Vitaceae*. In: Wu, Z.-Y., D.-Y. Hong & P. H. Raven (Eds.), *Flora of China*. Beijing and St. Louis, Mo.: Science Press and Missouri Botanical Garden Press, pp. 173–222.
- Chernetsky, M. & Weryszko-Chmielewska, E. (2008) Structure of *Kalanchoë pumila* Bak. leaves (Crassulaceae DC.). *Acta Agrobotanica*, 61, 11–24.
<https://doi.org/10.5586/aa.2008.029>
- Chu, Z.F. (2019) Genome size evolution of Vitaceae and phylogenetic relationships of *Nekemias*. Jishou University, Available from Cnki:
<https://kns.cnki.net/>
- Falcão, H.M., Medeiros, C.D., Silva, B.L.R., Sampaio, E.V.S.B., Almeida-Cortez, J.S. & Santos, M.G. (2015) Phenotypic plasticity and ecophysiological strategies in a tropical dry forest chronosequence: A study case with *Poincianella pyramidalis*. *Forest Ecology and Management*, 340, 62–69.
<https://doi.org/10.1016/j.foreco.2014.12.029>
- Fontenelle, G.B., Costa, C.G. & Machado, R.D. (2008) Foliar anatomy and micromorphology of eleven species of *Eugenia* L. (Myrtaceae). *Botanical Journal of the Linnean Society*, 116(2), 111–133.
<https://doi.org/10.1111/j.1095-8339.1994.tb00426.x>
- Fryns-Claessens, E. & Cotthem, W.R.J.V. (1973) A new classification of ontogenetic types of stomata. *Botanical Review*, 39(1), 71–138.
<https://doi.org/10.1007/BF02860071>
- Galet, P. (1967) *Recherches sur les methods d'identification et de classification des Vitacées tempérées. II Thèse, présentée a la Faculté des Sciences: Université de Montpellier*.
- Ishida, T., Kurata, T., Okada, K. & Wada, T. (2008) A Genetic Regulatory Network in the Development of Trichomes and Root Hairs. *Annual Review of Plant Biology*, 59(1), 365–386.
<https://doi.org/10.1146/annurev.arplant.59.032607.092949>
- Johnson, H.B. (1975) Plant pubescence: An ecological perspective. *The Botanical Review*, 41(3), 233–258.
<https://doi.org/10.1007/BF02860838>
- Kaya, A. & Koca, F. (2003) Comparative leaf anatomical studies of *Acinos* species (Labiatae) from Wkey. 23(5), 577–588.
<https://doi.org/10.1111/j.1756-1051.2003.tb00437.x>
- Klich, M.G. (2000) Leaf variations in *Elaeagnus angustifolia* related to environmental heterogeneity. *Environmental and Experimental Botany*, 44(3), 171–183.
[https://doi.org/10.1016/S0098-8472\(00\)00056-3](https://doi.org/10.1016/S0098-8472(00)00056-3)
- Liao, J.P., Zou, P. & Zhang, D.X. (2008) Leaf epidermal micromorphology of *Cercis* (Fabaceae: Caesalpinioideae). *Botanical Journal of the Linnean Society*, 158(3), 539–547.
<https://doi.org/10.1111/j.1095-8339.2008.00877.x>
- Linnaeus. (1753) *Species plantarum*. In (Vol. 1–2). Stockholm, Sweden. 616 pp.
- Lu, L.M., Wen, J. & Chen, Z.D. (2012) A combined morphological and molecular phylogenetic analysis of *Parthenocissus* (Vitaceae) and

- taxonomic implications. *Botanical Journal of the Linnean Society*, 168(1), 43–63.
<https://doi.org/10.1111/j.1095-8339.2011.01186.x>
- Meng, Y., Wang, J.J. & Nie, Z.L. (2016) Comparative morphology of leaf epidermis in 34 species of *Maianthemum* (Asparagaceae, Polygonateae) and their systematic significance. *Phytotaxa*, 275(2), 81–96.
<https://doi.org/10.11646/phytotaxa.275.2.1>
- Metcalf, C.R. & Chalk, L. (1988) *Anatomy of the dicotyledons: Systematic anatomy of leaf and stem, with a brief history of the subject* (2nd ed. Vol. 1). Oxford: Clarendon Press.
- Michaux, A. (1803) *Flora boreali-americana, sistens characteres plantarum quas in America septentrionali collegit et detexit Andreas Michaux* (Vol. v.1). Parisiis et Argentorati: apud fratres Levrault. 159 pp.
<https://doi.org/10.5962/bhl.title.330>
- Neto, I.L.D.C., Martins, F.M., Martins, M.L.L. & Silva, L.R.D.O. (2017) Comparative leaf anatomy of wild *Manihot* Mill. species (Euphorbiaceae) from Chapada Diamantina, Bahia, Brazil. *Nordic Journal of Botany*, 35(2), 207–219.
<https://doi.org/10.1111/njb.01324>
- Nie, Z.L., Sun, H., Manchester, S.R., Meng, Y., Luke, Q. & Wen, J. (2012) Evolution of the intercontinental disjunctions in six continents in the *Ampelopsis* clade of the grape family (Vitaceae). *BMC Evolutionary Biology*, 12, 17.
<https://doi.org/10.1186/1471-2148-12-17>
- Nitta, I. & Ohsawa, M. (1997) Leaf dynamics and shoot phenology of eleven warm-temperate evergreen broad-leaved trees near their northern limit in central Japan. *Plant Ecology*, 130(1), 71–88.
<https://doi.org/10.1023/A:1009735709258>
- Nurul-Aini, C.C., Noraini, T., Latiff, A., Amirul-Aiman, A.J., Ruzi, A.R. & Idris, S. (2014) Taxonomic significance of leaf micromorphology in some selected taxa of Acanthaceae (Peninsular Malaysia). *AIP Conference Proceedings*, 1614(1), 727–733.
<https://doi.org/10.1063/1.4895291>
- Rafinesque, C.S. (1838) *Sylva telluriana. mantis. synopt. new genera and species of trees and shrubs of North America, and other regions of the earth, omitted or mistaken by the botanical authors and compilers, or not properly classified, now reduced by their natural affinities to the proper natural orders and tribes*. Printed for the author and publisher: Philadelphia, pp. 86–89.
<https://doi.org/10.5962/bhl.title.22070>
- Ren, H., Lu, L.M., Soejima, A., Luke, Q., Zhang, D.X., Chen, Z.D. & Wen, J. (2011) Phylogenetic analysis of the grape family (Vitaceae) based on the noncoding plastid *trnC-petN*, *trnH-psbA*, and *trnL-F* sequences. *Taxon*, 60, 629–637.
<https://doi.org/10.1002/tax.603001>
- Ren, H., Pan, K.Y., Chen, Z.D., & Wang, R.Q. (2003) Structural characters of leaf epidermis and their systematic significance in Vitaceae. *Journal Systematic and Evolution*, 41(6), 531–544.
- Serna, L., & Martin, C. (2006) Trichomes: different regulatory networks lead to convergent structures. *Trends in Plant Science*, 11(6), 274–280.
<https://doi.org/10.1016/j.tplants.2006.04.008>
- Société Botanique De, F., & Centre National De La Recherche, S. (1886) Bulletin de la Société botanique de France. 33, 460.
- Soejima, A., & Wen, J. (2006) Phylogenetic analysis of the grape family (Vitaceae) based on three chloroplast markers. *American Journal of Botany*, 93(2), 278–287.
<https://doi.org/10.3732/ajb.93.2.278>
- Stace, C.A. (1965) *Cuticular studies as an aid to plant taxonomy*. In: *Bulletin of the British Museum (Natural History)*: Botany pp. 3–78.
- Stace, C.A. (1984) *The Taxonomic Importance of the Leaf Surface*. In: Heywood, V. H. & D. M. Moore (Eds.), *Current Concepts in Plant Taxonomy*. London: Academy Press, pp. 67–94.
- Trias-Blasi, A., John A.N. Parnell, & Trevor R.H. (2012) Multi-gene region phylogenetic analysis of the grape family (Vitaceae). *Systematic Botany*, 37(4), 941–950.
<https://doi.org/10.1600/036364412X656437>
- Ullah, F., Zafar, M., Ahmad, M., Shah, S.N., Razzaq, A., Sohail, A., Zaman, W., Çelik, A., Ayaz, A., & Sultana, S. (2018) A systematic approach to the investigation of foliar epidermal anatomy of subfamily Caryophylloideae (Caryophyllaceae). *Flora*, 246–247, 61–70.
<https://doi.org/10.1016/j.flora.2018.07.006>
- Wen, J. (1999) Evolution of eastern Asian and eastern North American disjunct distributions of flowering plants. *Annual Review of Ecology & Systematics*, 30(1), 421–455.
<https://doi.org/10.1146/annurev.ecolsys.30.1.421>
- Wen, J. (2007) *Vitaceae*. In: Kubitzki, K. (Ed.), *Flowering Plants Eudicots: Berberidopsidales, Buxales, Crossosomatales, Fabales p.p., Geraniales, Gunnerales, Myrtales p.p., Proteales, Saxifragales, Vitales, Zygophyllales, Clusiaceae Alliance, Passifloraceae Alliance*,

Dilleniaceae, Huaceae, Picramniaceae, Sabiaceae. Berlin, Heidelberg: Springer Berlin Heidelberg, pp. 467–479.

https://doi.org/10.1007/978-3-540-32219-1_54

- Wen, J., Boggan, J., & Nie, Z.L. (2014) Synopsis of *Nekemias* Raf., a segregate genus from *Ampelopsis* Michx. (Vitaceae) disjunct between eastern/southeastern Asia and eastern North America, with ten new combinations. *PhytoKeys*, 42, 11–19.
<https://doi.org/10.3897/phytokeys.42.7704>
- Wen, J., Lu, L.M., Nie, Z.L., Liu, X.Q., Zhang, N., Ickert Bond, S., Gerrath, J., Manchester, S.R., Boggan, J., & Chen, Z.D. (2018) A new phylogenetic tribal classification of the grape family (Vitaceae). *Journal of Systematics and Evolution*, 56(4), 262–272.
<https://doi.org/10.1111/jse.12427>
- Wen, J., Nie, Z.L., Soejima, A., & Meng, Y. (2007) Phylogeny of Vitaceae based on the nuclear GAI1 gene sequences'. *Canadian Journal of Botany-Revue Canadienne De Botanique*, 85(8), 731–745.
<https://doi.org/10.1139/B07-119>
- Wen, J., Xiong, Z.Q., Nie, Z.L., Mao, L.K., Zhu, Y.B., Kan, X.Z., Ickert-Bond, S.M., Gerrath, J., Zimmer, E.A., & Fang, X.D. (2013) Transcriptome sequences resolve deep relationships of the grape family. *PLoS ONE*, 8(9), e74394.
<https://doi.org/10.1371/journal.pone.0074394>
- Wiggers, F.H. (1780) *Primitiae Florae Holsaticae Litteris Mich. Frider. Bartschii Acad. Typogr., Kiel*, pp 1–112.
- Wilkinson, H.P. (1979) *The plant surface (mainly leaf)*. In: Metcalfe, C. R. & L. Chalk (Eds.), *Anatomy of the dicotyledons*. Oxford: Clarendon Press, pp. 97–165.
- Xia, Y. (2016) *Study of medicinal plants of Ampelopsis by DNA molecular identification and chemical composition analysis*. Hubei University of Chinese Medicine, Wuhan.
- Yan, A., Pan, J., An, L., Gan, Y., & Feng, H. (2012) The responses of trichome mutants to enhanced ultraviolet-B radiation in *Arabidopsis thaliana*. *Journal of Photochemistry and Photobiology B: Biology*, 113, 29–35.
<https://doi.org/10.1016/j.jphotobiol.2012.04.011>
- Zhang, N., Wen, J., & Zimmer, E.A. (2015) Congruent deep relationships in the grape family (Vitaceae) based on sequences of chloroplast genomes and mitochondrial genes via genome skimming. *PLoS ONE*, 10(12), e0144701.
<http://dx.doi.org/10.1371/journal.pone.0144701>