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A revised classification of leaf variegation types

Jian-Hang Zhang[a], Jin-Chu Zeng[a], Xiao-Mei Wang[a], Shui-Fei Chen[b], Dirk C. Albach[c,*], Hong-Qing Li[a,*]

[a] School of Life Sciences, East China Normal University, Shanghai 200241, China
[b] Research Center for Nature Conservation and Biodiversity / State Environmental Protection Scientific Observation and Research Station for Ecology and Environment of Wuyi Mountains/ State Environmental Protection Key Laboratory on Biosafety, Nanjing Institute of Environmental Sciences, Ministry of Ecology and Environment, Nanjing 210042, China
[c] Institut für Biologie und Umweltwissenschaften (IBU), Carl von Ossietzky-Universität Oldenburg, Carl von Ossietzky-Str. 9-11, 26111 Oldenburg, Germany

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ABSTRACT

Variegated leaf plants are a group of plants with stable patterns of differently colored leaf areas. The variously colored patches on the surface of the leaves have important biological functions in plant reproduction and adaptation to the environment. Apart from that, these plants have attracted interest as valuable ornamental plants. In this study, 1710 species with variegated leaves belonging to 78 families were investigated based on field-collected samples and previous literature, including transverse sections of 117 species. The macroscopic patterns of variegated leaves are highly diverse and can be distinguished as fishbone-shaped, blotched, V-shaped, spotted, striped, reticulate, and pinnate, with varying levels of diversity across different families and genera. We classified variegated leaves into five types according to the location, shape, color, and cross-sectional structure of the differently colored leaf areas. These are: I, chlorophyll type; II, air space type; III, epidermis type; IV, pigment type; and V, appendages type. Type II is the most common type, which is found in approximately 56% of all variegated leaf species, whereas type V is newly defined to accommodate the variegated leaves with colored, unequally distributed, multicellular outgrowths on the epidermis. Relationships between the diverse macroscopic patterns and the five structural types are discussed.

1. Introduction

Variegated leaves exhibit a mosaic of one or several non-green leaf areas. The diversity of patterns in variegated leaves (Wang and Xu, 2007; Hou, 2014) stirred an early interest in classifying different types of variegation. Hara (1957) was the first to study comprehensively the patterns of color patches among different species. His concept of leaf variegation excluded pathologically induced patterns (Hara, 1957). More recently, Sheue et al. (2012) focused on the developmental processes underlying variegation and defined variegated leaf plants as those with the presence of multiple colors on the leaf surface variously arranged as irregular spots or patches and regular patterns. Following the concepts proposed by Hara (1957) and Sheue et al. (2012), we define variegated leaves as those with color patterns formed by regular or irregular spots and patches, but excluding those with chlorophyll-deficient lesions caused by pathological reasons and those with variegated areas outside the leaf blades (e.g., petiolar variegations of *Amorphophallus*).

Variegated leaf plants are taxonomically diverse and geographically widely distributed. They are usually herbaceous or climbing, rarely shrubby or arboreal, mainly distributed in evergreen broad-leaved forests, with the highest species diversity under the canopy of tropical rain forests (Burtt, 1977; Givnish, 1990; Tsukaya, 2004). Several studies have suggested that leaf variegation plays a significant role in adaptation to abiotic factors, as well as in the avoidance of predation, and in reproduction (Sheue et al., 2012; Pao et al., 2014; Shelef et al., 2019). For example, studies on the photosynthetic system of variegated leaves of *Arum italicum* (Araceae) have shown that photosystem II in discolored areas of leaves maintains high activity under low light conditions, and the high photosynthetic efficiency of the discolored areas of leaves is closely related to their 2-layered palisade tissue, which is likely the result of their long-term adaptation to low light environment under the forest canopy (La Rocca et al., 2011; Wang et al., 2016b). Leaf variegation can reduce the damage from herbivores to the leaves of *Hydrophyllum virginianum* (Campitelli et al., 2008) and can mimic plain leaves with infestation of leaf-mining moth larvae to prevent moth oviposition

* Corresponding authors.
E-mail addresses: dirk.albach@uni-oldenburg.de (D.C. Albach), hqli@bio.ecnu.edu.cn (H.-Q. Li).

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to the leaves of *Caladium steudnerifolium* (Soltau et al., 2009). In some other cases, leaf variegation can defend leaves from large herbivores by camouflage or aposematism as forms of Batesian mimicry and Müllerian mimicry (Lev-Yadun, 2014; Lev-Yadun and Niemelä, 2017). The white patches of *Silybum marianum* serve as visual defense from herbivory, but also increase leaf interior temperature to enhance environmental adaptability (Lev-Yadun, 2003; Lev-Yadun, 2017; Shelef et al., 2019). Furthermore, the fungal-like 'mildew spots' of *Cypripedium fargesii* can attract pollinating insects (Ren et al., 2011).

Apart from evolutionary and ecological studies, also the genetic basis of leaf variegation has attracted scientific interest. Brown (1947) suggested that the V-shaped variegation of *Trifolium repens* is controlled by a series of allelic loci. Brewbaker (1955) reported that the spotless type of *T. repens* is recessive. Corkill (1971) further demonstrated that the red veins, diffuse red leaves, and red leaves of *T. repens* are regulated by R^m, R^{id} and R^l genes, respectively, and are inherited independently. Henny (1983) found that the leaf variegation of the genus *Aglaonema* is controlled by multiple alleles at a single locus. Li et al. (2001) found that the silver-green variegation and the lack of variegation of *Begonia rex* are genetically coupled traits, and their inheritance follows the Mendelian law of segregation.

Since a general scheme for the genetic regulation of leaf patterning is lacking and is unlikely to emerge soon, the classification of leaf variegation types continues to rely on leaf cell shape, as well as biochemical composition and distribution of discolored patches (Zhang et al., 2009; Sheue et al., 2012; Chen et al., 2017; Pao et al., 2020). Hara (1957: 87) classified leaf variegation into four types based on 55 species in 24 families in Japan: 1) 'chlorophyll' type, based on the deficiency of chlorophyll in the non-green area of the leaf; 2) 'air space' type, defined by the existence of intercellular air spaces just below the epidermis; 3) 'epidermis' type, based on the specific morphology of adaxial epidermal cells; and 4) 'pigment' type, defined by the accumulation of non-photosynthetic pigments that mask the green color of leaves. However, his work included only one sample of the 'epidermis' type. Pao et al. (2020) recently provided another example of the epidermal variegation caused by larger epidermal cells and thicker outer tangential cell walls. Further, Hara (1957) did not include species-rich groups of variegated leaf plants such as Marantaceae and Bromeliaceae. Lee (2007) suggested that the white areas were caused by tissues between veins without functioning chloroplasts, such as in *Sonerila margaritacea*, or along the primary vein, such as in *Fittonia albivenis*. However, Lee's argument is not entirely valid because the author did not consider the intercellular spaces around the palisade tissue cells of the main vein into account. La Rocca et al. (2011: 1392) reported that there was only one layer of palisade cells in the white areas and two layers in the green areas of leaves of *Arum italicum*, and thus, he proposed the 'variable palisade' type. According to the location of the non-green parts and whether the veins were related, leaf variegations of begonias were divided into 'the vein' type and 'the non-vein' type (Cui and Guan, 2013). Lev-Yadun (2014) proposed three additional types of white variegation: 1) 'the genetic chimera' type, in which variegation is caused by two kinds of cells with different genotypes in shoot apical meristems with one cell-type containing chloroplasts and the other not; 2) 'the unbalanced redox state of chloroplast' type, in which an unbalanced redox state in the photosynthetic electron transport chain predisposes the chloroplasts to photooxidation, consequently bleaching the leaves; and 3) 'the various biochemical modifications altered chloroplast' type, in which modifications include chloroplast development, structural change, and biochemical process. Chen et al. (2017) suggested the 'chloroplast' type and 'crystal' type, since they found that the size of chloroplasts in palisade cells and the accumulated crystal content in epidermal cells in the non-green area can be different from those in the neighboring areas. Additionally, considering the palisade cells and adjacent spongy tissue cells between the non-green and the green area, Chen et al. (2017) proposed that the 'variable palisade' type should be integrated into the 'upper mesophyll' type. Because the aforementioned studies on leaf variegation (Lee, 2007; La Rocca et al., 2011; Cui and Guan, 2013; Chen et al., 2017; Pao et al., 2020) only focused on a limited number of families and genera, the results do not suffice to improve Hara's (1957) classification system.

Fig. 1. Colors described according to RGB color model.

On the basis of previous typifications of variegated leaves, we aim to provide basic data for their genetic and evolutionary research, and for cultivating ornamental varieties of variegated leaf plants by carrying out morpho-anatomical observations and by providing an extensive species list of angiosperms with variegated leaves.

2. Materials and methods

According to our definition of variegated leaf plants (species with non-pathological color variegation on leaf blades), a species list was compiled based on information from field investigations and previous literature (e.g., Wu and Raven, 2003; Flora of North America Editorial Committee, 1993; Global Biodiversity Information Facility: www.gbif.org/; nomenclatural reference source: www.plantsoftheworldonline.org/). The list does not include plants with ever-colored leaves, seasonally colored leaves, pathological spots, or variegated plants obtained by artificial mutation. The macroscopic characteristics of variegated leaves, such as color, and shape, were recorded and classified.

Based on macroscopic observations, variegation patterns were preliminarily classified and plants with typical spots were selected for anatomical observation. Freehand sectioning was carried out on 49 species from 24 families, of which more than 10 species per leaf variegation type of Hara (1957) were included. Another 68 species from 31 families recorded in the literature (e.g., Du et al., 2018; Hara, 1957; Sud and Dengler, 2000) were also typified (Appendix). Fresh healthy leaves were selected for slice preparation. Three non-green areas and three green areas from the same leaf (or similar leaves from the same plant) per species were transversely sectioned. For leaves with small and scattered non-green patches, one non-green area and its adjacent green area (side by side) were free-hand sliced as a whole for parallel observation. If the non-green patches were large enough, the non-green area and the green area were free-hand sliced separately. Leaf shape, variegation position, boundary sharpening of the spots, and surface appendages (multicellular epidermal outgrowths, for example scales, trichomes, papillae, or prickles) were recorded using a digital camera and stereoscope (KEYENCE VHX-5000 Japan). We describe the variegation positions as along veins, interveinal, across main vein only, across main and lateral veins, at leaf margin, or at both sides of the main vein. The colors were described approximately as dark green, green, lime green, light green, green yellow, light lime, yellow, champagne yellow, white, pearl pink, light violet, purple, or dark red according to RGB color model (CIE, 1932; see Fig. 1). Color differences between non-green and green areas were assumed according to the chlorophyll content (low/normal, detected by naked eyes referring obvious color differences) or non-photosynthetic pigments (present/absent). Size, shape and number of epidermal cell layers, shape and arrangement of palisade cells, the quantity of chloroplasts, and distribution of non-photosynthetic pigments were examined using a NIKON DS-Ri2 microscope. Following Hara (1957), we classified leaf variegation mainly by the anatomy of

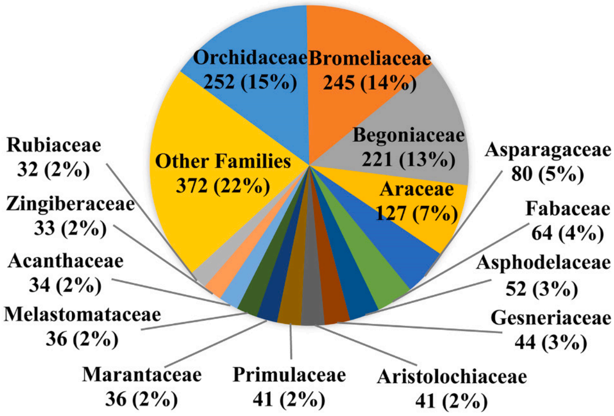

Fig. 2. Numbers and percentages of species with variegated leaves per family.

transverse sections, given consideration of the location, shape, and color of the spot area. For the unsampled variegated leaf plants, the variegation based on macroscopic characters such as shape, location, and color of the non-green patches, and reference to their taxonomical position.

3. Results

3.1. Number of species with variegated leaves in each family

A total of 1710 angiosperm species belonging to 356 genera and 78 families are reported here as having variegated leaves. Orchidaceae (252 species), Bromeliaceae (245 species), Begoniaceae (221 species), Araceae (127 species), and Asparagaceae (80 species) are the top five families with most species bearing variegated leaves, representing 54% of the total number of species investigated (Fig. 2). Among genera, *Begonia* (Begoniaceae) is the genus with most species (221 species) bearing variegated leaves (Appendix), followed by *Paphiopedilum* (Orchidaceae, 67 species), *Neoregelia* (Bromeliaceae, 62 species), *Trifolium* (Fabaceae, 41 species), *Asarum* (Aristolochiaceae, 38 species),

Fig. 3. Fishbone-shaped leaf variegation. **a**, *Impatiens wenshanensis* (II); **b**, *Rubus corchorifolius* (II); **c**, *Viola selkirkii* (II); **d**, *Gardneria multiflora* (II); **e**, *Breynia spatulifolia* (II); **f**, *Clematis finetiana* (II); **g**, *Euonymus fortunei* (II); **h**, *Dorstenia foetida* (II); **i**, *Arisaema erubescens* (II); **j**, *Pyrola calliantha* (II); **k**, *Gynostemma pentaphyllum* (II); **l**. *Reynoutria multiflora* (II); **m**, *Saxifraga stolonifera* (II, IV); **n**, *Youngia erythrocarpa* (IV). Roman numbers II and IV refer to the anatomical classification described in section 3.3. (see below).

Fig. 4. Blotched (white and/or colored) leaf variegation. **a**, *Thladiantha maculata* (II); **b**, *Akebia trifoliata* (II); **c**, *Peperomia argyreia* (II); **d**, *Polygala latouchei* (II); **e**, *Schisandra propinqua* (II); **f**, *Tripterospermum chinense* (II); **g**, *Hoya sigillatis* (II); **h**, *Maesa perlarius* (II); **i**, *Cissus wenshanensis* (II, IV); **j**, *Pilea sinofasciata* (II); **k**, *Actinidia lanceolata* (II, IV); **l**, *Asarum forbesii* (II); **m**, *Saururus chinensis* (II); **n**, *Kaempferia rotunda* (II); **o**, *Hepatica nobilis* (II); **p**, *Pueraria montana* (II); **q**, *Smilax guiyangensis* (II, IV); **r**, *Strobilanthes auriculata* (II, IV); **s**, *Podophyllum versipelle* (IV); **t**, *Pelargonium* × *hybridum* (IV); **u**, *Maranta leuconeura* (III, IV); **v**, *Colocasia esculenta* (IV); **w**, *Trapa natans* (IV); **x**, *Hygroryza aristata* (IV). Roman numbers II, III, and IV refer to the anatomical classification described in section 3.3. (see below). Photos **s, x** by Jianmei Wu and **q** by Xiaocong Ke.

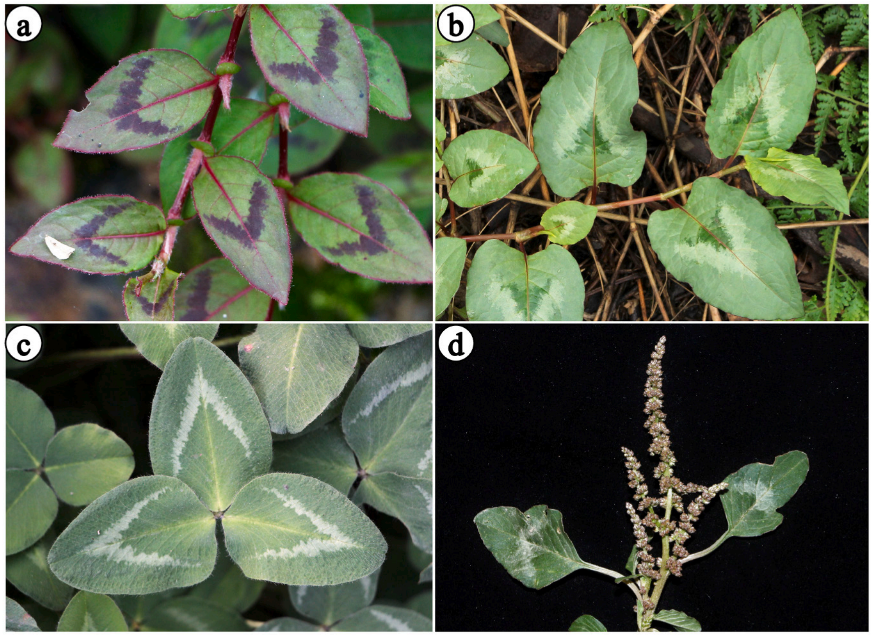

Fig. 5. V-shaped leaf variegation. **a**, *Persicaria capitata* (IV); **b**, *Persicaria chinensis* (II, IV); **c**, *Trifolium pratense* (II); **d**, *Amaranthus viridis* (II). Roman numbers II and IV refer to the anatomical classification described in section 3.3. (see below).

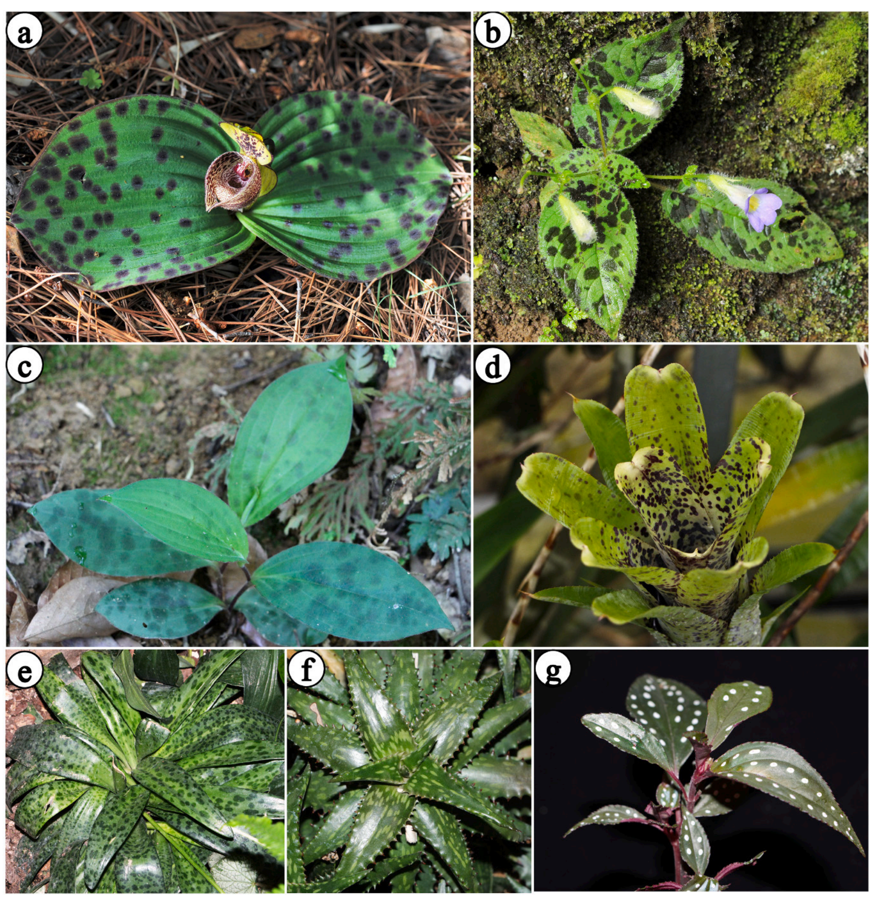

Fig. 6. Spotted leaf variegation. **a**, *Cypripedium margaritaceum* (IV); **b**, *Henckelia pumila* (IV); **c**, *Tricyrtis macropoda* (IV); **d**, *Neoregelia ampullacea* (IV); **e**, *Ledebouria socialis* (IV); **f**, *Aloe jucunda* (IV); **g**, *Sonerila cantonensis* (II). Roman numbers II and IV refer to the anatomical classification described in section 3.3. (see below). Photo **a** by Jianmei Wu.

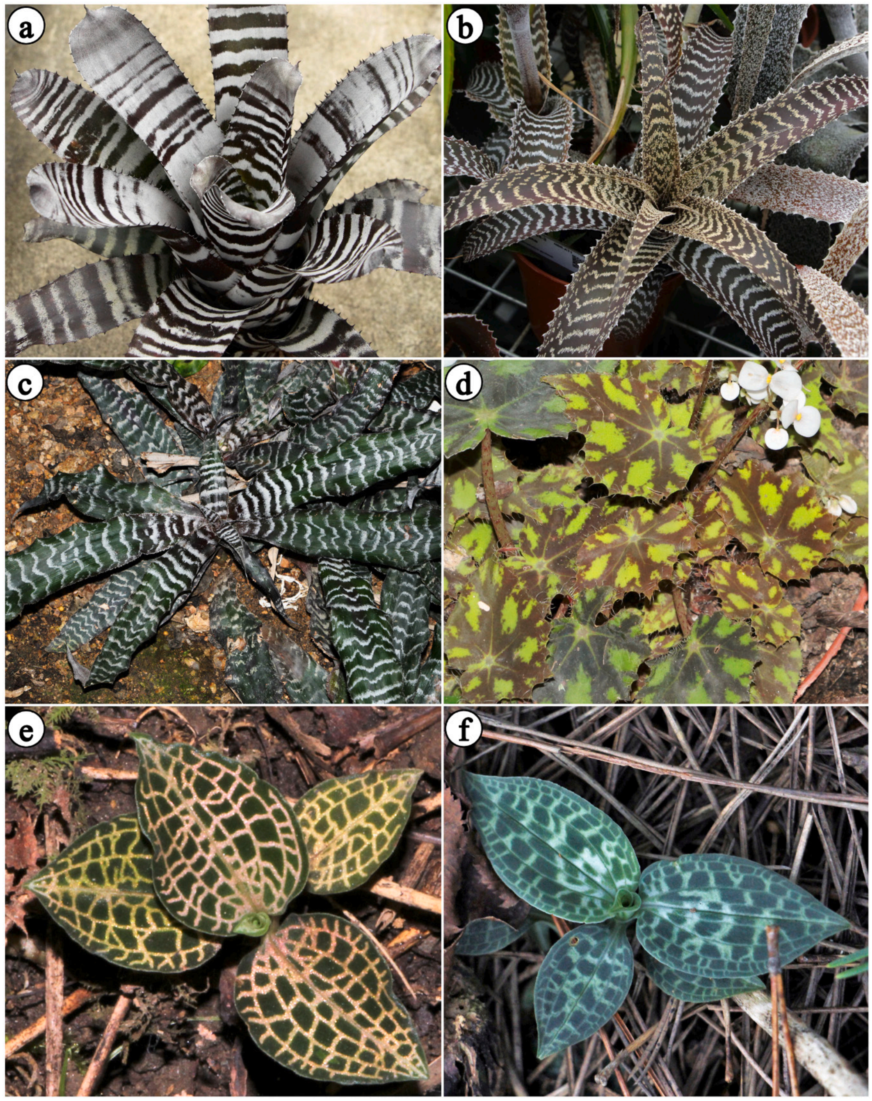

Fig. 7. Striped (a-c) and reticulate (d-f) leaf variegation. Striped: **a**, *Aechmea chantinii* (V); **b**, *Orthophytum gurkenii* (V); **c**, *Cryptanthus zonatus* (V). Reticulate: **d**, *Begonia bowerae* (IV); **e**, *Goodyera biflora* (II); **f**, *Goodyera schlechtendaliana* (II). Roman numbers II, IV, and V refer to the anatomical classification described in section 3.3. (see below).

Aechmea (Bromeliaceae, 36 species), *Goeppertia* (Marantaceae, 28 species), *Persicaria* (Polygonaceae, 27 species), *Ledebouria* (Asparagaceae, 25 species), *Gasteria* (Asphodelaceae, 23 species), and *Mussaenda* (Rubiaceae, 23 species). These eleven genera account for approximately 35% of the total variegated leaf species (Appendix).

3.2. Macroscopic leaf variegation patterns

By observing the macroscopic characters of leaf variegation, size, shape and color of leaf patches were found to be highly diverse. Variegations of most species were generally white and belonged to one of seven distinguishable patterns. However, some species displayed intermediate or mixed patterns, with a combination of colors (e.g., *Saxifraga stolonifera* (Fig. 3m), *Actinidia lanceolata* (Fig. 4 k), *Begonia pseudodryadis, Smilax guiyangensis* (Fig. 4q), and *Trillium maculatum*). The seven types are:

Fishbone-shaped: The pattern of variegation is conspicuous along the main vein, gradually narrower along the primary lateral veins, and fades away from the veins. Three hundred and forty-four species exhibit this pattern, e.g., *Impatiens wenshanensis* (Fig. 3a).

Blotched: The pattern of variegation is blurry and irregular, often located between primary lateral veins or in some species located across the veins. Eight hundred and eighty-one species exhibit this pattern. Variegations within this group were either white (387 species, Fig. 4 a-q) or colored (494 species, Fig. 4 r-x). White and dark red blotches appeared in *Smilax guiyangensis* (Fig. 4 q), whereas fishbone-shaped and the blotched pattern co-occur in *Saxifraga stolonifera* (Fig. 3 m).

V-shaped: Non-green areas form an inverted "V", such as in *Persicaria capitata* and forty-nine other species (Fig. 5 a-d).

Spotted: Non-green areas show rounded outline, such as in *Cypripedium margaritaceum*. Two hundred and seventy-five species exhibit this pattern (Fig. 6 a-g).

Striped: Non-green areas are arranged transversely or longitudinally in stripes on the leaves, such as in *Aechmea chantinii* and *Haworthiopsis reinwardtii*. Forty-one species (Fig. 7 a-c) exhibit this pattern.

Reticulate: Non-green areas interwoven along the main vein, the first lateral veins and the second lateral veins. One hundred and fifteen species exhibit this pattern; e.g. *Begonia bowerae* and several species of *Goodyera* (Fig. 7 d-f).

Pinnate: Large and small alternate patches forming a penniform pattern. Sixteen species exhibit this pattern; mostly in Marantaceae and Zingiberaceae, such as in some species of *Ctenanthe* and *Goeppertia*(Fig. 8

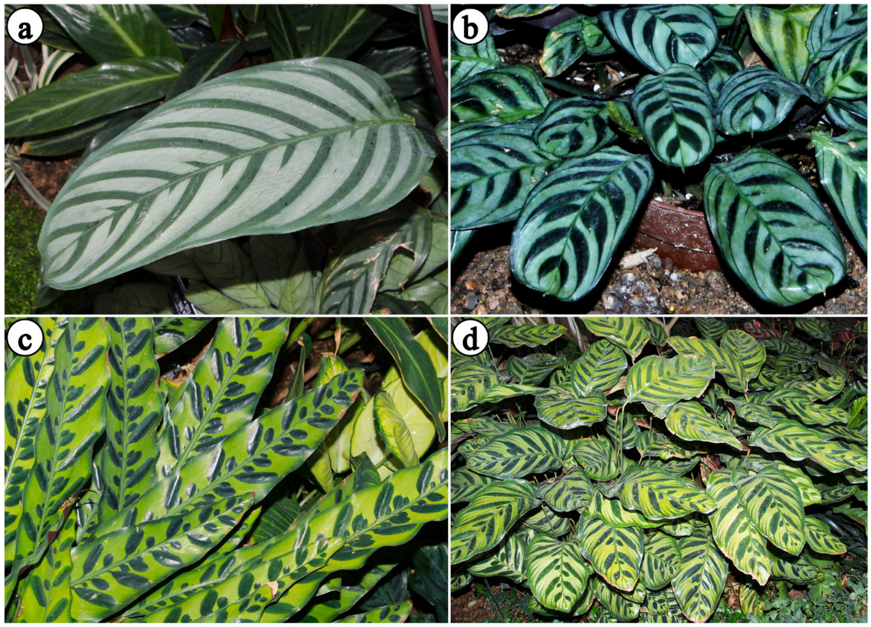

Fig. 8. Pinnate leaf variegation. **a**, *Ctenanthe setosa* (II); **b**, *Ctenanthe burle-marxii* (I, III); **c**, *Goeppertia insignis* (I); **d**, *Goeppertia makoyana* (I). Roman numbers I, II, and III refer to the anatomical classification described in section 3.3. (see below).

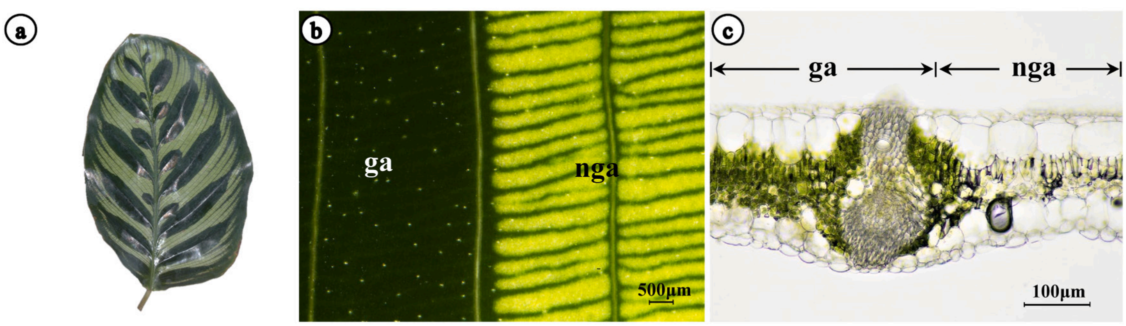

Fig. 9. Comparative leaf structure between green areas and non-green areas of 'chlorophyll' type in *Goeppertia makoyana*. **a**, variegated leaf; **b**, magnified portion of the leaf; **c**, transverse section; **ga**, green area; **nga**, non-green area.

a-d).

3.3. Anatomical classification of leaf variegation

Based on examination of transverse sections and previous records (Hara, 1957; Zhang et al., 2009; La Rocca et al., 2011; Sheue et al., 2012; Cui and Guan, 2013; Chen et al., 2017), 117 representative species of variegated leaf plants were classified according to anatomical characters. The anatomy of epidermal cells, epidermal appendages (multicellular epidermal outgrowths), palisade tissue, chlorophyll content (low/normal), and presence of non-photosynthetic pigments and crystals were compared between the non-green and green areas. According to these traits, we propose the following five types:

Chlorophyll type (Type I): The chlorophyll content in the chloroplasts of discolored areas is usually obviously less than that in green areas. No intercellular air spaces occur between the epidermal and palisade cells or among palisade cells at non-green areas. An example is *Goeppertia makoyana*, where the discolored areas are almost absent of chlorophyll and are light lime, but chlorophyll is present in normal amounts in dark-green areas (Figs. 8 d, 9). Both the mesophyll cells in discolored area and green area have identical chloroplasts, and also the shape and size of the adaxial epidermal cells are identical in these two areas (Fig. 9 c). Externally, the discolored parts of the leaves of this type are usually light green or yellowish green, and blotched or pinnate on the leaf surface (Fig. 8 b-d). Seven species (*Ctenanthe burle-marxii, Eupatorium chinense, Goeppertia insignis, Goeppertia makoyana, Pseudowintera colorata, Stenotaphrum secundatum* and *Zanthoxylum bungeanum*) observed exhibit this type, and voucher information is at the Appendix. Leaves of this type mainly belong to species of Araceae, Bromeliaceae, Marantaceae, and Rubiaceae (Appendix).

Air space type (Type II): Variegation in this type is caused by conspicuous intercellular spaces between the epidermal and palisade cells and among palisade cells at non-green areas. Differences between non-green and green areas can also be found in sizes, shapes, and arrangement of mesophyll cells, or in number of cell layers of palisade tissue. Examples are *Persicaria chinensis* (Figs. 5 b, 10 d-g) and *Sonerila cantonensis* (Figs. 6 g, 10 a-c). The palisade cells in non-green areas are spherical and loosely arranged (Fig. 10 c, g), whereas in green areas they are cylindrical and tightly arranged (Fig. 10 c, f). The non-green areas are whitish and there is no significant difference in the shape and size of the adaxial epidermal cells between non-green and green areas. Additional non-photosynthetic pigments may or may not occur in the green areas or the non-green areas (Fig. 10 c, f, g, j). The above mentioned species have variegations in the leaves caused by intercellular spaces between veins, whereas some other species contain intercellular spaces along the veins. For example, in *Gardneria multiflora* (Figs. 3 d, 10 h-j),

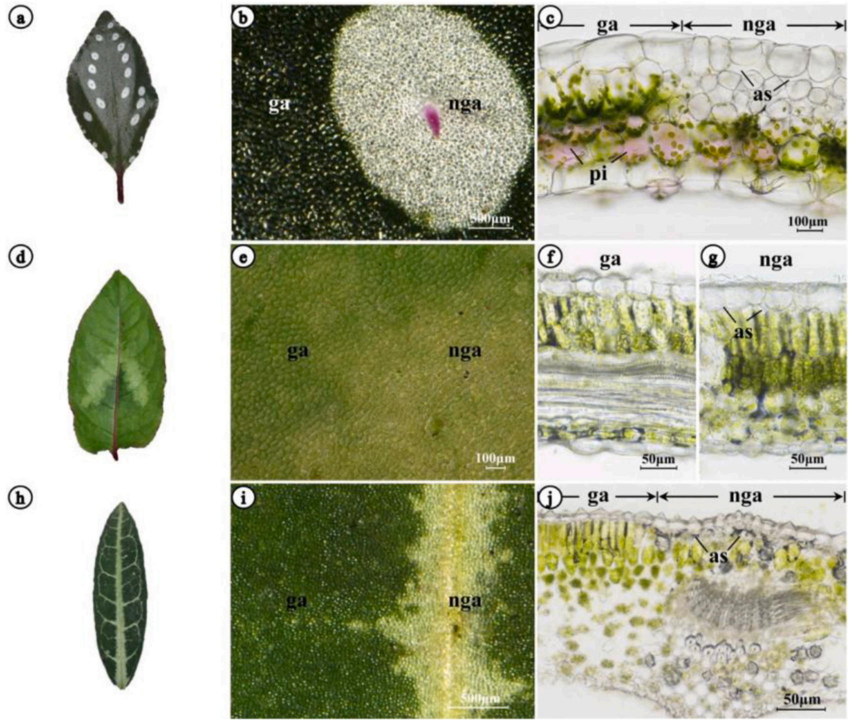

Fig. 10. Comparative leaf structures between green areas and non-green areas of 'air space' type. **a-c**, *Sonerila cantonensis*; **d-g**, *Persicaria chinensis*; **h-j**, *Gardneria multiflora*; **a, d, h**, variegated leaf; **b, e, i**, magnified portion of the leaf; **c, f, g, j**, transverse sections; **as**, air space; **ga**, green area; **nga**, non-green area; **pi**, pigments.

the cell shapes of palisade tissue around vascular bundles in non-green areas are different from those in green areas owing to the cell differentiation and organization of vascular tissue. Its macroscopic leaf variegation pattern is fishbone-shaped (Figs. 3 d, 10 h, i). *Asarum forbesii* (Fig. 4 l), *Pyrola calliantha* (Fig. 3 j), *Trifolium pratense* (Fig. 5 c), and *Tripterospermum chinense* (Fig. 4 f) all have intercellular spaces in the palisade tissues. Macroscopically, the non-green parts of the leaves of this type can be white and fishbone-shaped, blotched, V-shaped or spotted (Figs. 3 a-m, 4 a-q, 5 b-d, 6 f, g). Seventy-nine species observed belong to this type (Table 1), which is taxonomically widely distributed in Araceae, Orchidaceae, Piperaceae, Ranunculaceae, Begoniaceae, Fabaceae, Melastomataceae, Primulaceae, Smilacaceae, and some other families (Appendix).

Epidermis type (Type III): In this type, the cell size, shape, and thickness of outer tangential cell walls, or the presence of hypodermal cells differ in regions of variegation. Variegation is only associated to the epidermal and hypodermal cells, excluding the unequally distributed multicellular appendages outside the adaxial surface of the epidermal cells. Taking *Goeppertia zebrina* as the first example, the variegations are light green and dark green (Fig. 11 a, b). The epidermal cells are basically the same size and shape in the two differently colored areas and there is no visible intercellular space among palisade cells, though palisade cells in light green area are thinner than those in the darker areas (Fig. 11 c, d). Its hypodermal cells in light green areas are significantly transversely extended and resulting in the reduction of palisade tissue, or inversely, the reduction of palisade tissue provides place for hypodermal cells extending (Fig. 11 d). Similar patterns among hypodermal and palisade cells are found in *Cornukaempferia aurantiiflora* and some other species (Table 2). The second example is *Maranta leuconeura* (Figs. 4 u, 11 e-h). Its epidermal cells are flat in whitish areas but papillose in green areas, and its hypodermal cells in whitish areas are larger than that in green areas (Fig. 11 g, h). These traits cause the variegation, but red pigments in the green areas further increase the difference in color (Fig. 11 h). The third example, *Haworthiopsis fasciata*, presents bundled hypodermal cells in non-green areas whereas hypodermal cells are lacking in green area (Fig. 11 k). The discolored areas of this type are usually white or light green blotched (Figs. 4 u, 8 b). Eleven species observed belong to this type. Some of the species of Marantaceae, Oxalidaceae, Melastomataceae, and Zingiberaceae have the epidermis type variegation (Appendix).

Pigment type (Type Ⅳ): The accumulation of non-photosynthetic pigments, such as anthocyanins and flavonoids, results in the formation of leaf variegation in this type. The leaves are colored, but not white or yellowish-green. Examples are *Aeschynanthus longicaulis* (Fig. 12 a-c) and *Colocasia esculenta* (Figs. 4 v, 12 d-f). The surfaces of the non-green areas are dark red (Fig. 12 a, d), and no differences were observed in the size and shape of epidermal cells or mesophyll cells between both areas. Purple-red pigments accumulate in the abaxial epidermal cells of *Aeschynanthus longicaulis* (Fig. 12 c), whereas dark red pigments gather in the palisade tissue of *Colocasia esculenta* (Fig. 12 f). Macroscopically, the non-green parts of the leaves of this type are blotched, V-shaped, spotted or reticulate (Figs. 4 q-x, 5 a, 6 a-e, 7 d). Thirty-one species observed (Table 3) and about 39% of the listed variegated leaf species in Appendix correspond to this type.

Appendages type (Type V): Variegations are caused by unequally distribution of colored multicellular outgrowths on the adaxial epidermis of leaves. For example, there are white and dense multicellular scales on the epidermis of whitish areas in *Hohenbergia correia-arauji* and forming zebra stripe shapes, but no other discernible differences between epidermal cells of dark red areas and whitish areas (Fig. 13). Macroscopically the non-green parts of the leaves of this type are white stripes on the leaf surface (Fig. 7 a-c). Four species (*Aechmea chantinii, Cryptanthus zonatus, Hohenbergia correia-arauji* and *Orthophytum gurkenii*) observed belong to this type (voucher data found in Appendix). This kind of variegated leaves is only found in some genera of Bromeliaceae (Appendix).

Some variegated leaves may possess two different variegation types, partly or entirely overlaid, or adjacent to form multicolor areas (Table 1-3). For example, the marked leaf area of *Maranta leuconeura* (Figs. 4 u, 11 e-h) has papillose epidermal cells, and these cells also contain non-photosynthetic pigments (Fig. 11 g, h). *Persicaria chinensis* (Figs. 5 b, 10 d-g) and *Erythronium dens-canis* (La Rocca et al., 2014) possess white

Table 1
Anatomical characters on transverse sections of ‘air space’ type leaves

species	air space		palisade tissue cell shape		voucher information/references
	non-green area	green area	non-green area	green area	
Actinidia kolomikta	+	-	spherical/cp-	columnar	Wang et al., 2016a
Actinidia polygama	+	-	- -	- -	Hara, 1957
Ainsliaea apiculata	+	-	- -	- -	Hara, 1957
Akebia quinata	+	-	- -	- -	Hara, 1957
Akebia trifoliata	+	-	- -	- -	Hara, 1957
Amana erythronioides	+	-	- -	- -	Hara, 1957
Arisaema aequinoctiale	+	-	- -	- -	Hara, 1957
Arum italicum	+	-	ovoid	columnar	La Rocca et al., 2011
Asarum blumei	+	-	- -	- -	Hara, 1957
Asarum caudigerum	+	-	spherical	columnar	Zhen Zhang, LV20180021
Asarum fauriei	+	-	- -	- -	Hara, 1957
Asarum geophilum	+	-	spherical	columnar	Yanwen Liu, LV20170074
Asarum tamaense	+	-	- -	- -	Hara, 1957
Begonia × albopicta	+	-	- -	- -	Hara, 1957
Begonia aptera	+	-	ovoid	columnar	Pao et al., 2020
Begonia deliciosa	+	-	spherical	columnar	Du et al., 2018
Begonia formosana	+	-	spherical	columnar	Sheue et al., 2012
Begonia gulinqingensis	+	-	spherical	columnar	Du et al., 2019
Begonia handelii	+	-	spherical	columnar	Du et al., 2018
*Begonia pseudodryadis**	+	-	spherical/cp-	columnar	Du et al., 2018
Begonia rex	+	-	ovoid	columnar	Hara, 1957; Zhang et al., 2009
Blastus cochinchinensis	+	-	spherical/cp-	columnar	Chen et al., 2017
Breynia spatulifolia	+	-	ovoid	columnar	Danyun Xu, LV20190016
Calanthe argenteostriata	+	-	ovoid	columnar	JH Zhang, LV20180046
Chrysosplenium flagelliferum	+	-	- -	- -	Hara, 1957
Chrysosplenium macrostemon	+	-	- -	- -	Hara, 1957
Circaea alpina	+	-	- -	- -	Hara, 1957
Cirsium nipponicum	+	-	- -	- -	Hara, 1957
Clematis apiifolia	+	-	- -	- -	Hara, 1957
Clematis finetiana	+	-	ovoid	columnar	JH Zhang et al., LV20180009
Clematis terniflora	+	-	- -	- -	Hara, 1957
*Cornukaempferia aurantiiflora**	+	-	spherical	columnar	JH Zhang, LV20180024
Crocus vernus	+	-	- -	- -	Hara, 1957
Ctenanthe setosa	+	-	ovoid	columnar	JH Zhang, LV20190017
Cyclamen hederifolium	+	-	ovoid	columnar	Klančnik et al., 2016
Cyclamen persicum	+	-	ovoid	columnar	JH Zhang, LV20180047
Cyclamen purpurascens	+	-	- -	- -	Hara, 1957
Dumasia truncata	+	-	- -	- -	Hara, 1957
*Erythronium dens-canis**	+	-	spherical	columnar	La Rocca et al., 2014
*Erythronium japonicum**	+	-	- -	- -	Hara, 1957
Gardneria multiflora	+	-	spherical/cp-	columnar	JH Zhang et al., LV20180029
Goeppertia concinna	+	-	spherical	columnar	JH Zhang, LV20180030
Goeppertia majestica	+	-	spherical	columnar	JH Zhang, LV20180051
Goeppertia roseopicta	+	-	spherical	columnar	JH Zhang, LV20180049
Goodyera hachijoensis	+	-	- -	- -	Hara, 1957
Goodyera schlechtendaliana	+	-	ovoid	columnar	HuaiZhen Tian et al., LV20170073
Goodyera similis	+	-	- -	- -	Hara, 1957
Hepatica nobilis	+	-	- -	- -	Hara, 1957
*Kaempferia rotunda**	+	-	ovoid	triangular	JH Zhang, LV20170026
*Ludisia discolor**	+	-	spherical	columnar	Poobathy et al., 2018
Mucuna pruriens	+	-	- -	- -	Hara, 1957
Nervilia nipponica	+	-	ovoid	columnar	Pao et al., 2020
Ornithogalum gussonei	+	-	- -	- -	Hara, 1957
Oxalis acetosella	+	-	ovoid	columnar	Pao et al., 2020
Paphiopedilum concolor	+	-	ovoid	columnar	Pao et al., 2020
*Paphiopedilum malipoense**	+	-	spherical	columnar	HuaiZhen Tian et al., LV20170072
Peperomia argyreia	+	-	- -	- -	Hara, 1957
*Persicaria chinensis**	+	-	spherical	columnar	Liangyun Zhou, LV20180019
Pilea cadierei	+	-	ovoid	columnar	JH Zhang, LV20180048
Pyrola calliantha	+	-	ovoid	columnar	JH Zhang et al., LV20180039
Pyrola renifolia	+	-	- -	- -	Hara, 1957
Reynoutria multiflora	+	-	spherical	triangular	JH Zhang, LV20170006
Saururus chinensis	+	-	spherical/cp-	columnar	JH Zhang, LV20190026
*Saxifraga stolonifera**	+	-	ovoid	triangular	JH Zhang, LV20170004
Schismatoglottis calyptrata	+	-	ovoid	columnar	Tsukaya et al., 2004
Silybum marianum	+	-	- -	- -	Hara, 1957
Smilax bracteata	+	-	ovoid	columnar	Pao et al., 2020
Smilax sieboldii	+	-	- -	- -	Hara, 1957
*Sonerila cantonensis**	+	-	spherical/cp-	columnar	JH Zhang, LV20190013
Sonerila obliqua	+	-	spherical/cp-	columnar	Chen et al., 2017
Tradescantia zebrina	+	-	- -	- -	Hara, 1957
*Tricyrtis hirta**	+	-	- -	- -	Hara, 1957
Trifolium pratense	+	-	loosely columnar	neatly columnar	JH Zhang, LV20180011

(*continued on next page*)

Table 1 (*continued*)

species	air space		palisade tissue cell shape		voucher information/references
	non-green area	green area	non-green area	green area	
Trifolium repens	+	-	loosely columnar	neatly columnar	JH Zhang, LV20170001
*Tripterospermum chinense**	+	-	loosely columnar	neatly columnar	JH Zhang et al., LV20170053
Valeriana hsui	+	-	ovoid	columnar	Pao et al, 2020
Viola japonica	+	-	spherical	columnar	Zhen Zhang, LV20180022
Viola tokubuchiana	+	-	- -	- -	Hara, 1957
Viola yunnanfuensis	+	-	spherical	columnar	JH Zhang, LV20170063

Note: +, with visible air spaces; -, no visible air space; - -, missing data; *, with non-photosynthetic pigments in mesophyll cells; cp-, lower quantity of chloroplasts in non-green than in green areas.

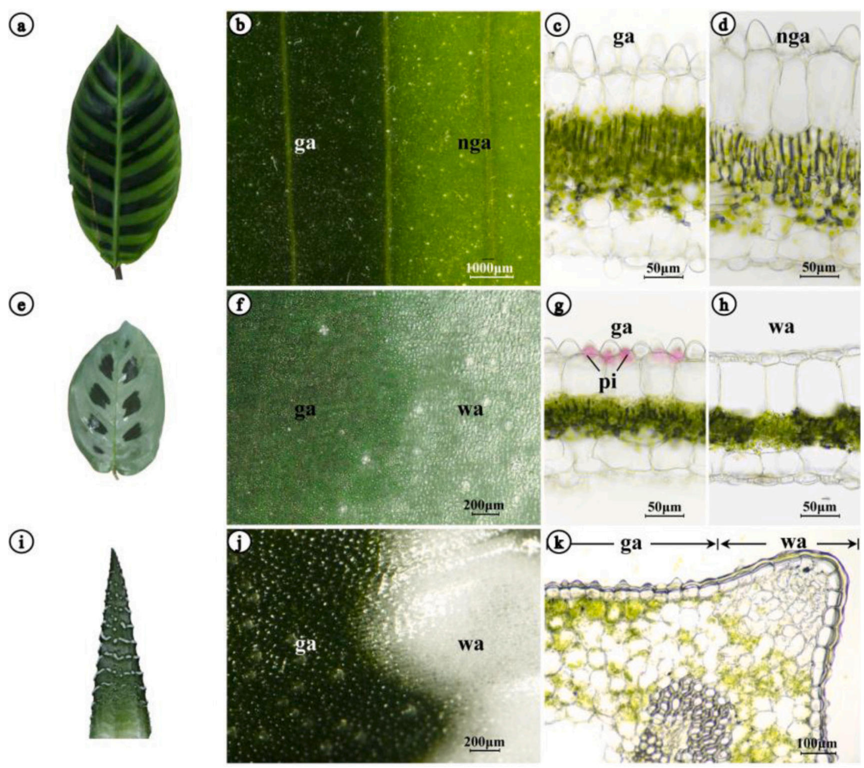

Fig. 11. Comparative leaf structures between green areas and non-green areas of 'epidermis' type. **a-d**, *Goeppertia zebrina*; **e-h**, *Maranta leuconeura*; **i-k**, *Haworthiopsis fasciata*; **a, e, i**, variegated leaf; **b, f, j**, magnified portion of the leaf; **c, d, g, h, k**, transverse sections; **ga**, green area; **nga**, non-green area; **pi**, pigments; **wa**, whitish area.

Table 2
Anatomical traits on transverse sections of 'epidermis' type leaves

species	epidermal cell shape		hypodermal cell shape		voucher information/references
	non-green area	green area	non-green area	green area	
Actinidia kolomikta	elliptical	oblong	no	no	Wang et al., 2016a
Blastus cochinchinensis	irregular	oblong	no	no	Chen et al., 2017
Cornukaempferia aurantiiflora	oblong	triangular	cubic	oblong	JH Zhang, LV20180024
Ctenanthe burle-marxii	oblong	oblong	cubic	oblong	JH Zhang, LV20190014
Goeppertia majestica	oblong	oblong	cubic	oblong	JH Zhang, LV20180051
Goeppertia zebrina	triangular	triangular	cubic	oblong	JH Zhang, LV20180045
Haworthiopsis fasciata	oblong	oblong	cubic, bundled	no	JH Zhang, LV20200012
Maranta leuconeura	triangular	oblong	cubic	cubic	JH Zhang, LV20180042
Oxalis debilis	elliptical	oblong	no	no	Pao et al., 2020
Oxalis latifolia	elliptical	oblong	- -	- -	Hara, 1957
Sonerila obliqua	elliptical	oblong	no	no	Chen et al., 2017

Note: - -, missing data; no: no hypodermal cells between epidermis and palisade cells.

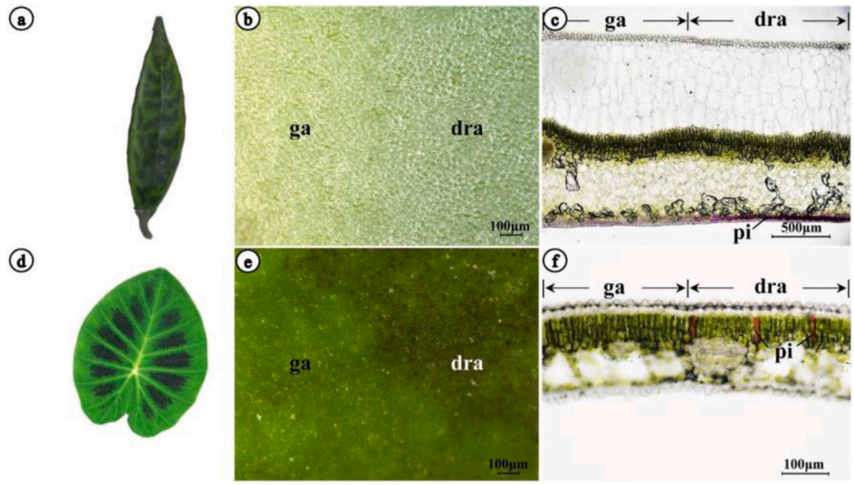

Fig. 12. Comparative leaf structures between green areas and non-green areas of 'pigment' type. **a-c**, *Aeschynanthus longicaulis*; **d-f**, *Colocasia esculenta*; **a, d**, variegated leaf; **b, e**, magnified portion of the leaf; **c, f**, transverse section; **dra**, dark red area; **ga**, green area; **pi**, pigments.

spots (air space type) and dark red spots (pigment type) on the same leaf simultaneously. Fourteen observed species present two types of variegation on the same leaf.

4. Discussion

4.1. Perspectives on the classification of leaf variegation

Our classification into five anatomical types of leaf variegation has been derived from a comprehensive analysis of previous studies (Hara, 1957; La Rocca et al., 2011; Cui and Guan, 2013; Chen et al., 2017) combined with our data from additional anatomical observations. Four of these types match closely to those defined by Hara (1957), although we provide more details on these types. Particularly, the identification of the 'chlorophyll' type (type I), 'air space' type (type II) and 'epidermis' type (type III) have been carefully defined by adding evidence from color of non-green areas, anatomical data, or comparison of closely related species. Thus, in concordance with Hara (1957) we continued to use his names, although they may not always be ideal. For example, we cannot be sure that all the intercellular spaces of the 'air space' type are indeed filled with air. Apart from that, 'chlorophyll' type has been used differently by Hara (1957) and Chen et al. (2017) and there may be some confusion caused by continuing to use this name. Nevertheless, since our 'chlorophyll' type matches closely the description of Hara (1957) we consider continuity in names more appropriate. Hara (1957) subdivided each of his variegation types in vein type, partial type, and entire type, but this was not practical since many observed cases demonstrate that these subpatterns can overlap. Therefore, we rejected further subdivisions but established a separate system allowing recognition by macroscopic investigations, which is convenient for easier application in the field (Fig. 3-8). Meanwhile, we found that for about 80% of the genera with variegated leaf species, variegation types are genus-specific (see Appendix), and that those genera with more than one kind of variegation type are species-rich genera, for example *Begonia* (Doorenbos et al., 1998), *Aechmea* (Sass and Specht, 2010), and *Goeppertia* (Borchsenius et al., 2012). This implies that the specific type of variegation is constrained to some extent by taxon-specific traits. However, in general, variegations seem to be more affected by leaf growth stage (Hara, 1957; La Rocca et al., 2014; Wang et al., 2016a) and environmental conditions (Mwafongo et al., 2017).

The 'appendages' type is formed by unequal aggregation of appendages on the adaxial epidermis and is not affected by the shape or size of epidermal cells. Functionally, epidermal appendages are believed to reduce transpiration, protect the leaf against ultraviolet radiation, or reduce damage from herbivory by vertebrates and insects (Kang et al., 2010; van Houten et al., 2013; Andrade et al., 2017; Kariyat et al., 2017; Zhang et al., 2018), and thus they are different from epidermal cells. Therefore, we treated unequally aggregated appendages as a new type. Uniformly distributed hairs, scales, or wax can also cause changes in leaf color, but there is no variegation presented on the leaf blade (Lee, 2007) and thus should not be considered here.

The structural basis of the 'air space' type variegation is the formation of intercellular lacunae beneath the epidermis. Hara (1957) explained in detail the causes for the formation of intercellular spaces and proposed 'air space' type. He revealed that in the process of leaf development, the growth of palisade cells could not keep pace with the growth of the epidermal cells, and large intercellular spaces are formed (Hara,1957). Therefore, light passing through these spaces causes distinct reflection and scattering, which is different from that of the areas without large intercellular spaces. Our anatomical observation of *Gardneria multiflora* (Figs. 3 d, 10 h-j) confirmed that 'the along the primary vein' type (Lee, 2007) and 'the vein' type (Cui and Guan, 2013) are identical to the 'air space' type. La Rocca et al. (2011) observed intercellular spaces in one layer of the palisade cells and proposed the 'variable palisade' type (integrated into the 'upper mesophyll' type by Chen et al., 2017), which is also a typical structure of the 'air space' type variegation. In these cases, the intercellular spaces exist both beneath the epidermis and between palisade cells.

Reduction in the size or number of chloroplasts can also form variegations (white spots). Chen et al. (2017) defined this as 'chloroplast' type. However, it is difficult to differentiate this type from his own 'upper mesophyll' type (palisade cells of white areas colorless and spongy cell-like, but with functional chloroplasts), since the formation of intercellular spaces in palisade tissue and a decrease in chloroplast number in the discolored area often co-occur (Hara, 1957; Zhang et al., 2009; La Rocca et al., 2011; Chen et al., 2017; Fig. 10 c, f, g, j). Moreover, studies of *Arabidopsis thaliana* indicated that the palisade cells of variegated mutants *var3* and *anu10-1* displayed abnormal shape, loose arrangement, obvious intercellular spaces and smaller chloroplasts compared to the wild type (Næsted et al., 2004; Casanova-Sáez et al., 2014), suggesting that chloroplast development is directly related to palisade cell development. Therefore, the 'chloroplast' type and 'upper mesophyll' type sensu Chen et al. (2017) are considered here to be equivalent to the 'air space' type.

In some plants with fishbone-shaped and white or grayish-white color variegations (Figs. 3 a-m, 10 h-j), chloroplasts are relatively rare in cells above and adjacent to vascular bundles, palisade cells are smaller, and intercellular spaces exist below the epidermis and between

Table 3
Anatomical traits on transverse sections of 'pigment' type leaves

species	non-photosynthetic pigments		air space		voucher information/ references
	non-green area	green area	non-green area	green area	
Acer distylum	#	ǂ	- -	- -	Hara, 1957
Aeschynanthus longicaulis	#	ǂ	-	-	JH Zhang et al., LV20180032
Begonia bowerae	#	ǂ	-	-	JH Zhang, LV20190011
Begonia pseudodryadis	#	ǂ	+	-	Du et al., 2018
Begonia variegata	#	ǂ	-	-	Du et al., 2018
Colocasia esculenta	#	ǂ	-	-	JH Zhang, LV20190018
Corylus sieboldiana	#	ǂ	- -	- -	Hara, 1957
Drimiopsis maculata	#	ǂ	-	-	JH Zhang, LV20180050
Erythronium dens-canis	#	ǂ	+	-	La Rocca et al., 2014
Erythronium japonicum	#	ǂ	+	-	Hara, 1957
Euphorbia hirta	#	ǂ	-	-	JH Zhang et al., LV20180031
Euphorbia maculata	#	ǂ	-	-	JH Zhang, LV20170003
Henckelia pumila	#	ǂ	-	-	JH Zhang et al., LV20180017
Hydrangea hydrangeoides	#	ǂ	- -	- -	Hara, 1957
Ledebouria revoluta	#	ǂ	-	-	Mwafongo et al., 2017
Ludisia discolor	#	ǂ	+	-	Poobathy et al., 2018
Maranta leuconeura	ǂ	#	-	-	JH Zhang, LV20180042
Neoregelia ampullacea	#	ǂ	-	-	JH Zhang, LV20190008
Pelargonium inquinans	#	ǂ	- -	- -	Hara, 1957
Persicaria chinensis	#	ǂ	+	-	Liangyun Zhou, LV20180019
Persicaria debilis	#	ǂ	- -	- -	Hara, 1957
Polygonatum lasianthum	#	ǂ	- -	- -	Hara, 1957
Persicaria filiformis	#	ǂ	-	-	JH Zhang et al., LV20180007; Hara, 1957
Persicaria longiseta	#	ǂ	- -	- -	Hara, 1957
Pseudowintera colorata	#	ǂ	-	-	Cooney et al., 2012
Saxifraga stolonifera	#	ǂ	+	-	JH Zhang, LV20170004
Smilax china	#	ǂ	- -	- -	Hara, 1957
Tricyrtis hirta	#	ǂ	+	-	Hara, 1957
Tricyrtis macropoda	#	ǂ	-	-	JH Zhang et al., LV20180054
Vriesea fosteriana	#	ǂ	-	-	JH Zhang, LV20190003
Vriesea saundersii	#	ǂ	-	-	JH Zhang, LV20190004

Note: #, present; ǂ, absent; +, with visible air spaces; -, no visible air space; - -, missing data.

palisade cells (Fig. 10 j). Here, the fading color is mainly caused by the scarcity of chloroplasts (due to intercellular spaces and small palisade cells) but not by the lack of chlorophyll in the chloroplasts. Thus, these variegations are here considered to belong to the 'air space' type rather than to the 'chlorophyll' type. Lee (2007) proposed that the 'along-the-primary-vein' type is caused by disfunctional chloroplasts, but he did not address the function of intercellular spaces in the leaf variegation generation and did not provide a detailed description on this feature. The white forms of 'inter-vein part' type and 'middle vein and intervein part' type variegations in *Begonia* from Cui and Guan (2013) likely are equivalent to the 'air space' type.

Since the 'chlorophyll type' is strictly related to palisade tissue and chlorophyll formation, it conforms to the 'chlorophyll' type described by Hara (1957; not sensu Chen et al. 2017). As to the differences between our 'chlorophyll' and 'air space' types, the latter can be distinguished from the former by the presence of intercellular spaces in the discolored areas, whereas in the former, the quantity of chloroplasts is the same in both green and discolored areas but the chlorophyll content within a chloroplast differs between these two areas (Fig. 9, 10). The variation in chlorophyll content of a chloroplast is believed to be genetically regulated (Wu, 2013) and may be influenced by the environment (Stöckel, 2011).

The 'epidermis' type proposed by Hara (1957) is characterized by the size and shape of epidermal cells. The single representative species *Oxalis martiana* Zucc. bears only one layer of epidermal cells. The ratio of the mean volume of epidermal cells in discolored parts to that in green parts is 1 to 0.34 (Hara, 1957). The presence of a hypodermis and the variation of size and shape of compound epidermal cells (Fig. 11 a-d) also result in variegations. Furthermore, the thickness of outer tangential walls of epidermal cells seems to influence leaf variegation (Pao et al., 2020). *Haworthiopsis fasciata* represents a particular example of this type, since white areas are formed by patches of hypodermal cells (Fig. 11). This is an important amendment to the 'epidermis' type concept of Hara (1957).

The ability to produce chloroplasts can also be restricted to specific cell lines in a leaf, which is called a genetic chimera. The distribution pattern of cell lines, their growth direction, and number of cell divisions in the apices result in variegation patterns (Tilney-Bassett, 1986.; Li and He, 2005; Lev-Yadun, 2014). In the non-green areas, the shapes of palisade cells and chloroplasts in the leaf differs from those in the green areas to form the leaf variegation (Bae et al., 2000; Xia et al., 2008). Since the concept of the 'genetic chimera' type has been proposed from the perspective of genetic engineering rather than natural variegation, it is not reflected in our system of five types.

Another mechanism causing variegation is the production of crystals. Crystals are found widely across algae, ferns, and seed plants (Arnott and Pautard, 1970; Prychid, 1999; Horner et al., 2012). Based on the presence of crystals in non-green areas but not in green areas, Chen et al. (2017) proposed the 'crystal' type. However, several studies revealed that intercellular spaces are required for the presence of crystals (Horner, 2012; Su et al., 2019). In addition, the formation of crystals in plants is often affected by seasonal variation, climate, growth stage, and regional condition (Wu et al., 2016). Therefore, we do not consider the production of crystals a distinct variegation type.

Considering the diversity of macroscopical and anatomical characters of leaf variegations, it is no surprise that variegated leaf species are taxonomically diverse (78 families, 356 genera, 1710 species confirmed). Moreover, variegated leaf plants often grow as sciophyte in forest understories with reduced light intensity (Chen, 2015). These shade-tolerant plants do not only need to adapt to shady environment but also to patchy but strong light-penetrating gaps in the canopy (canopy rift) (Lee, 2007; Poobathy et al., 2018), and, therefore, have advantages in adapting to this kind of environment. Thus, sciophyte-rich plant families such as Begoniaceae, Bromeliaceae, Orchidaceae, and Araceae, and genera such as *Asarum, Goeppertia*, and *Ledebouria* possess most species with variegated leaves.

4.2. A revised, extended list of variegated leaf plants

In general, variegations of the 'chlorophyll' type (Type I) often have yellowish-green blotched or light-green pinnate shaped appearances; those variegations of the 'air space' type (Type II) correspond to white

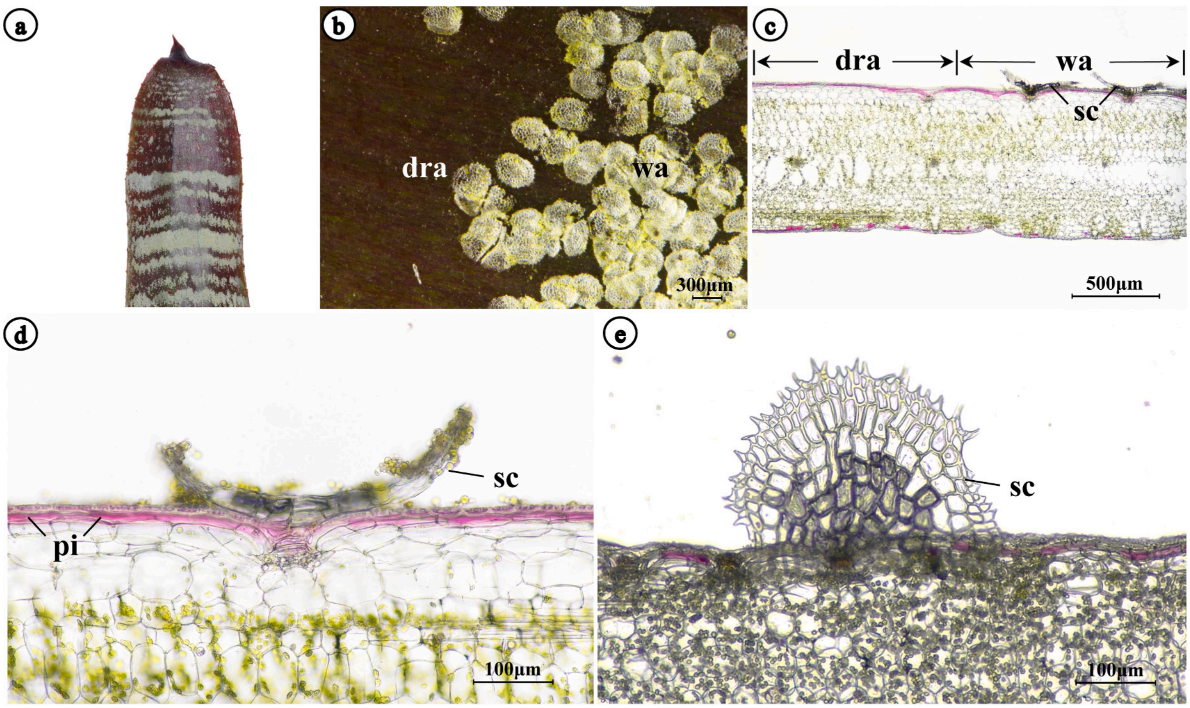

Fig. 13. Comparative leaf structure between green areas and non-green areas of 'appendages' type. **a-e**, *Hohenbergia correia-arauji*; **a**, variegated leaf; **b**, magnified portion of the leaf; **c**, transverse section; **d, e**, magnified portion of scales; **dra**, dark red area; **pi**, pigments; **sc**, scales; **wa**, whitish area.

fishbone-shaped, blotched, spotted, or V-shaped patterns; those of the 'epidermis' type (Type III) often exhibit blotched shapes (both colorful blotches and some instances of white blotches); those of the 'pigment' type (Type IV) commonly have leaves with fishbone-shaped, spotted, or blotched patterns, with red, dark-red, purple-black, or other colors; and, finally, those of the 'appendages' type (Type V) usually have white, striped shapes.

Based on the macroscopic morphological characteristics and observations of the transverse leaf sections of representative groups, we assembled a list of 1710 species having variegated leaves. Among them, 78 species belong to type I, 956 species belong to type II, 28 species belong to type III, 667 species belong to type IV, and 34 species belong to type V. A total of 66 species displayed two types of variegation, simultaneously. However, 12 spotted plants were not classified owing to insufficient understanding of their leaf structures (Appendix).

Author contributions

Hong-Qing Li and Jian-Hang Zhang designed the experiment. Jian-Hang Zhang, Xiao-Mei Wang, Jin-Chu Zeng and Shui-Fei Chen collected the samples. Jian-Hang Zhang and Jin-Chu Zeng performed the experiment and analyzed the data. Hong-Qing Li, Dirk C. Albach, Xiao-Mei Wang and Jian-Hang Zhang wrote the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

Declaration of Competing Interest

None.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.flora.2020.151703.

Appendix

Appendix: Leaf variegation types of variegated leaf angiosperms

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