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First report of cork-warts on a leaf of *Aristolochia* (Aristolochiaceae) and its systematic implication

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Abstract: Many species have cork-warts on their leaf lamina as an extra-taxonomic trait. The current research is the first to document the occurrence of cork warts in the Aristolochiaceae family, which have not been previously reported. The study's overarching goal is to provide taxonomic evidence of cork-warts in *Aristolochia* by documenting their occurrence, structure, and origin. Accordingly, examinations of the micro-morpho-anatomy of the leaves of *Aristolochia indica* L. and *Aristolochia ringens* Vahl. were used to assess the presence of cork-warts by using paradermal and transverse views of the leaf lamina. *A. indica* has an ordinary epidermal origin of corkwarts, but *A. ringens* contains d-stomata type origin of cork-warts. Therefore, corkwarts are seen as an extra taxonomic trait that may be used to distinguish between different species of *Aristolochia*.

Keywords: Leaf, Micro-morpho-anatomy, Systematics, Taxonomy

Introduction

The five genera *Saruma* Olive, *Asarum* L., *Aristolochia* L., *Thottea* Rottboll, and *Isotrema* Rafinesque make up the family Aristolochiaceae (Neinhuis *et al.* 2005). There are nearly 500 species of *Aristolochia* around the globe (udhakaran, 2016). Ravikumar *et al.* (2014) and Borah *et al.* (2019) reported about 20 *Aristolochia* species in India. The Indian Himalayas and sub-Himalayan areas are home to all these species, with some even making it to neighbouring nations. Although most of species in this genus are vines or lianas (Borah *et al.*, 2019), several species grow as herbs or even shrubs. *Aristolochia indica* L. and *Aristolochia ringens* Vahlare perennial climbers with many therapeutic uses.

Patterns of cuticular ornamentation, trichome type and stomatal type, foliar lenticels, and the presence of cork-warts are anatomical diagnostic features found in the leaves (Metcalfe & Chalk, 1950; Rasche & Kovar-Eder, 2009). According to Joffily and Vieira (2010), cork-warts are defined by suberin-encrusted regions that create layers of juxtaposed rectangular cells. These layers emerge from sequential tangential divisions and produce concentric structures. Furthermore, the cork-warts that develop on mature leaves have a practical purpose by facilitating gas exchange (Evans and Bromberg 2010). Metcalfe and Chalk (1950, 1979) and Solereder (1908) reported the presence of cork-warts in 21 families of dicotyledonous plants. Gnetaceae (Pagoda *et al.*, 2015), Celastraceae (Joffily& Vieira, 2010), Lauraceae (Vaz *et al.*, 2018), Rhizophoraceae (Duke & Bunt, 1979), Loranthaceae, and Myristicaceae have all been the subject of corkwart-related research in recent years.

Poulsen (1875), Bachmann (1880), Keller (1890), and Matteucci (1897) observed and described cork-wart formations for the first time which were often comparable to the lenticels seen on stems. The presence of corkwarts on the epidermis of leaves is a significant taxonomic feature since

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it is exclusive to certain plant families, varies in structure, and occurs less often. Stace (1965, 1966) and Den Hartog and Baas (1978) also addressed the use of cork-wart as a phylogenetic and systematic diagnostic trait. When cork-warts form because of damage, whether from fungus (Stace 1965; Buijsen 1995), insects (Baas 1975), or other causes, they are not of taxonomic significance, if they originated by stomata, glands in the epidermis, or the fall of trichomes (Farooqui, 1982; Metcalfe & Chalk, 1950; Stace, 1965) they contain taxonomic value.

The first work on the classification and description of four distinct types of cork-wart in two species of *Eucalyptus* was done by Farooqui (1982). In the present study presence of cork warts on the foliar lamina of two *Aristolochia* species has been discussed for the first time, along with their structure and origin, using paradermal and transverse sectional investigations of leaf micro-morpho-anatomy. The results of this research could help establish corkwarts as a diagnostic taxonomic trait for use in future taxonomic treatments to define the family's boundaries.

Materials and Methods

Fresh mature leaf samples of *A. indica* and *A. ringens* were collected from the Medicinal Plants Garden, Regional Ayurveda Research Institute, Pune. Voucher specimens for cross-verification and authentication of the species were prepared and the same has been deposited in the Herbarium at Regional Ayurveda Research Institute, Pune. Accession no. of Herbarium specimens are as follows.

1. *Aristolochia indica* L. (JNAMPGH - 15132)

2. *Aristolochia ringens* Vahl. (JNAMPGH -15554)

(i) Micro-morphological studies

Mature leaves were washed and the epidermis were peeled off by hand and stained with haematoxylin and safranin stain. After staining the leaf epidermises these were dehydrated through different grades of alcohol *i.e.* 10-100%. Dehydrated epidermises were then cleaned in clove oil and xylene (1:1) for 10 minutes. Finally, these were mounted on microscopic slides using Canada balsam. The photographic documentation and micromorphological data were recorded using an optical microscope (Olympus BX43) equipped with a digital camera.

(ii) Anatomical studies

The fresh leaves of *Aristolochia* species under study were re-hydrated following the method described by Smith & Smith (1942). After that, the material was cooled down at room temperature. The samples were sectioned by hand to obtain cross-sections of the middle portion of the leaf blade, using a razor blade. The standard laboratory procedure was used for making permanent slides. The sections were stained with Delafield's haematoxylin and safranin as per standard laboratory procedures. Stained sections were dehydrated in graded ethyl alcohol (10% -100%) and then cleared with clove oil and xylene before making permanent slides. The analysis and photographic documentation of anatomical data were made using an optical microscope (Olympus BX43) equipped with a digital camera.

Results

(i) Micro-morphological analysis

Epidermal cells

Upper and lower periclinal walls of the epidermis were slightly convex in both *A. indica* (Fig. 2d) and *A. ringens* (Fig. 4d). Cells were penta-, hexa- to polygonal, irregular to polygonal in shape and arranged irregularly. The upper epidermal cells usually had straight anticlinal walls in both species. Lower anticlinal walls of the epidermis were undulate V-shaped in *A. indica* (Fig. 1d) where as it was undulate U-shaped in *A. ringens* (Fig. 3d). In *A. indica*, the epidermis was striated or wrinkled with radiating cuticular striation at some places (Fig. 1b).

Cork-warts

The cell walls were found to be thickened and thickly cutinized. Cork-warts were located on both the adaxial and abaxial leaf surfaces in *A. indica* and *A. ringens.* The cork-warts observed in the upper epidermis of *A. indica* are simple types that originate from anticlinal divisions in radially arranged ordinary epidermal cells (Fig. 1a &b) without containing any idioblast associated with it (Fig. 2d) but cork warts present on lower surface are similar to *A. ringens* containing idioblast in mesophyll cells associated with

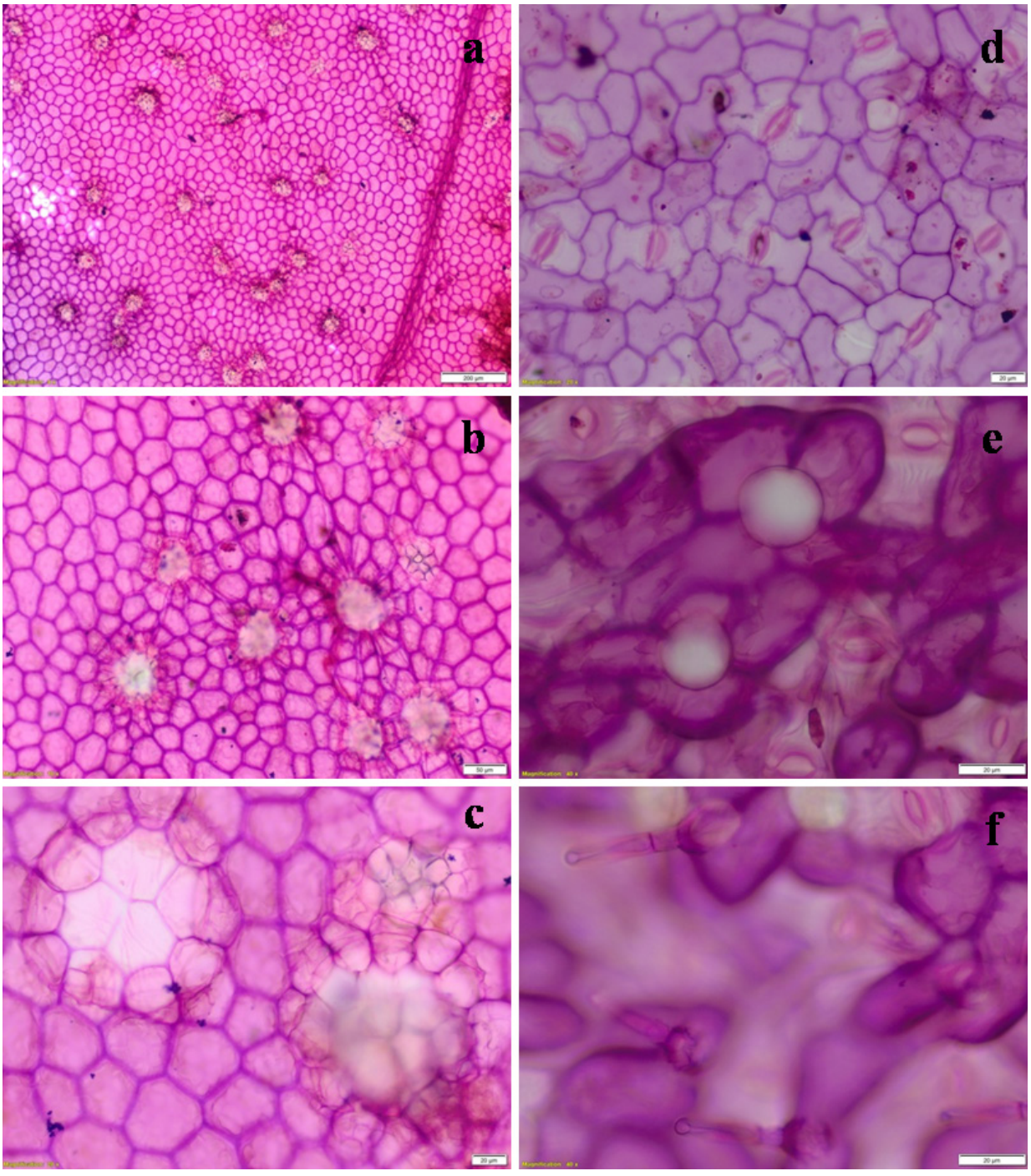

Fig. 1. *Aristolochia indica* L. **a** & **b**. Distribution and structure of cork warts on upper epidermis; **c.** Origin of corkwarts from anticlinal division in ordinary upper epidermal cells; **d.** Presence of anomocytic stomata and undulated V-shaped anticlinal walls on lower epidermis; **e**. Structure and development of corkwarts associated with idioblasts in lower epidermis; **f**. Presence of uniseriate multicellular hook like trichome on lower epidermis.

epidermal pore (Fig. 2b). Cork-warts observed in *A. ringens* are stomata-type warts, since they originated from the tangential divisions in the peripheral cells of D-type stomata (Fig. 3a &c). *A. ringens* have similar types of cork warts on both upper and lower epidermises associated with idioblasts located just below the epidermises (Fig. 3b & f). Some of the idioblast cells of *A. ringens* associated with cork warts deposited with yellow-coloured drops or substances while some of the idioblas twere empty (Fig. 4e). In cross sections of the leaf blade, we observed that cork-warts could also be derived from epidermal cells covering idioblasts. In this case, these cork-warts appeared to have been produced after anticlinal and periclinal divisions in epidermal cells located above the idioblasts.

Stomatal complex

Hypostomatic leaves were found in both the species of the genus the *Aristolochia*. Two types of stomata were recognized anisocytic and anomocytic. The most commontype of stomatal complex in the taxa investigated was anomocytic (Fig. 1d). Epidermal cells of the stomatal complex have a smooth surface in *A. ringens* whereas cuticular radiating striae extended as a lateral wing on either side of stomatal aperture is present in *A. indica* (Fig. 1e).

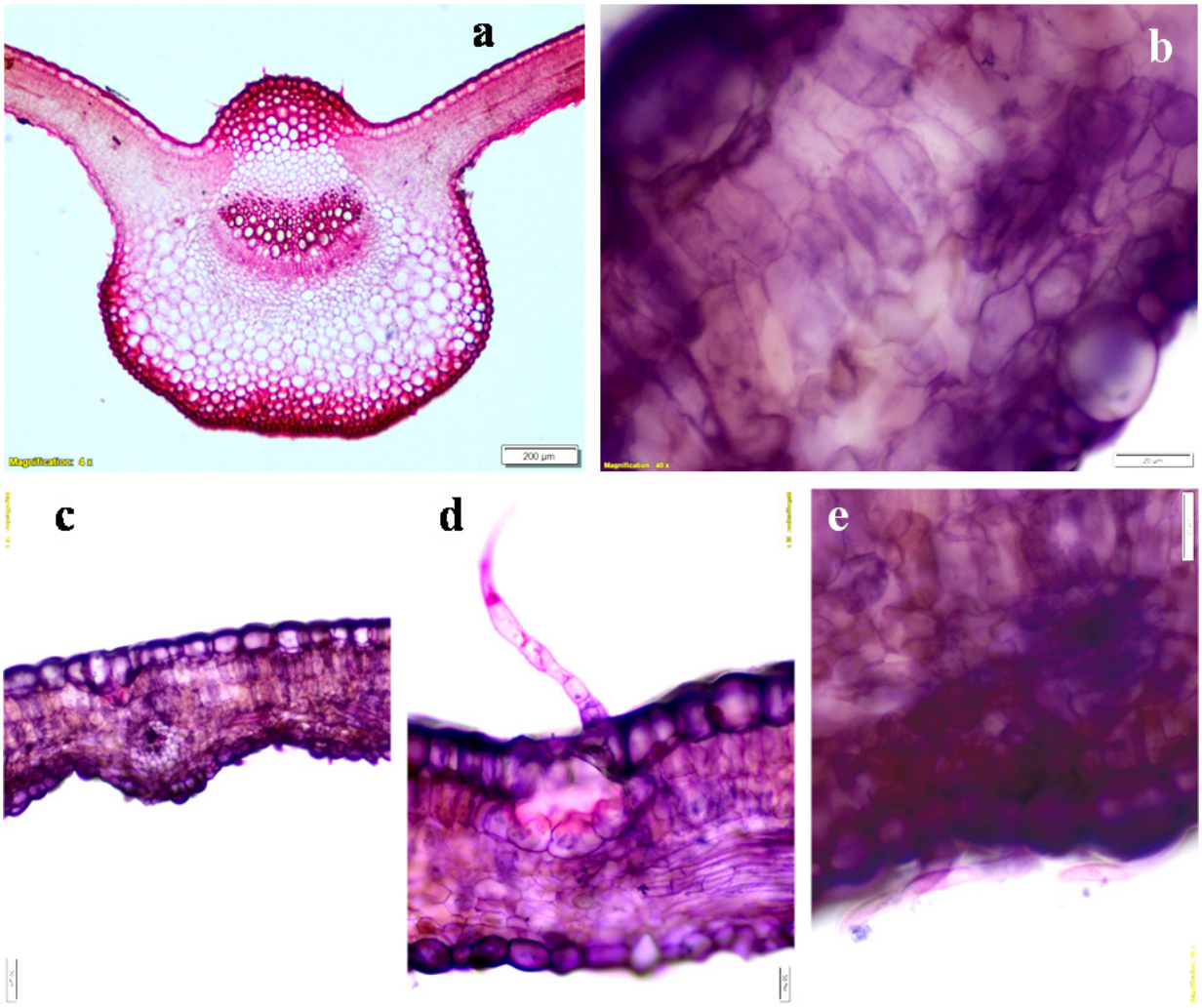

Fig. 2. *Aristolochia indica* L.: **a.** Cellular organization in midrib and lamina region; **b.** Palisade tissue in two layers and idioblast associated with corkwarts of lower epidermis; **c.** convex walls of upper epidermal cells and arrangement of palisade and spongy tissues; **d.** Presence of multicellular uniseriate trichome and corkwart cavity without idioblast on upper epidermis; **e.** presence of unseriate multicellular hook like trichome on lower epidermis.

Trichomes

Trichomes differ in *A. ringens* from *A. indica*. Uniseriate or multicellular hook like trichomes were present only on the lower-epidermis of the leaf in *A. indica* (Fig. 1f). Upper epidermis is smooth rarely containing any trichome except midrib region (Fig. 2a). A few uniseriate multicellular hook like trichomes are present on the upper surface of *A. ringens* (Fig. 4a).

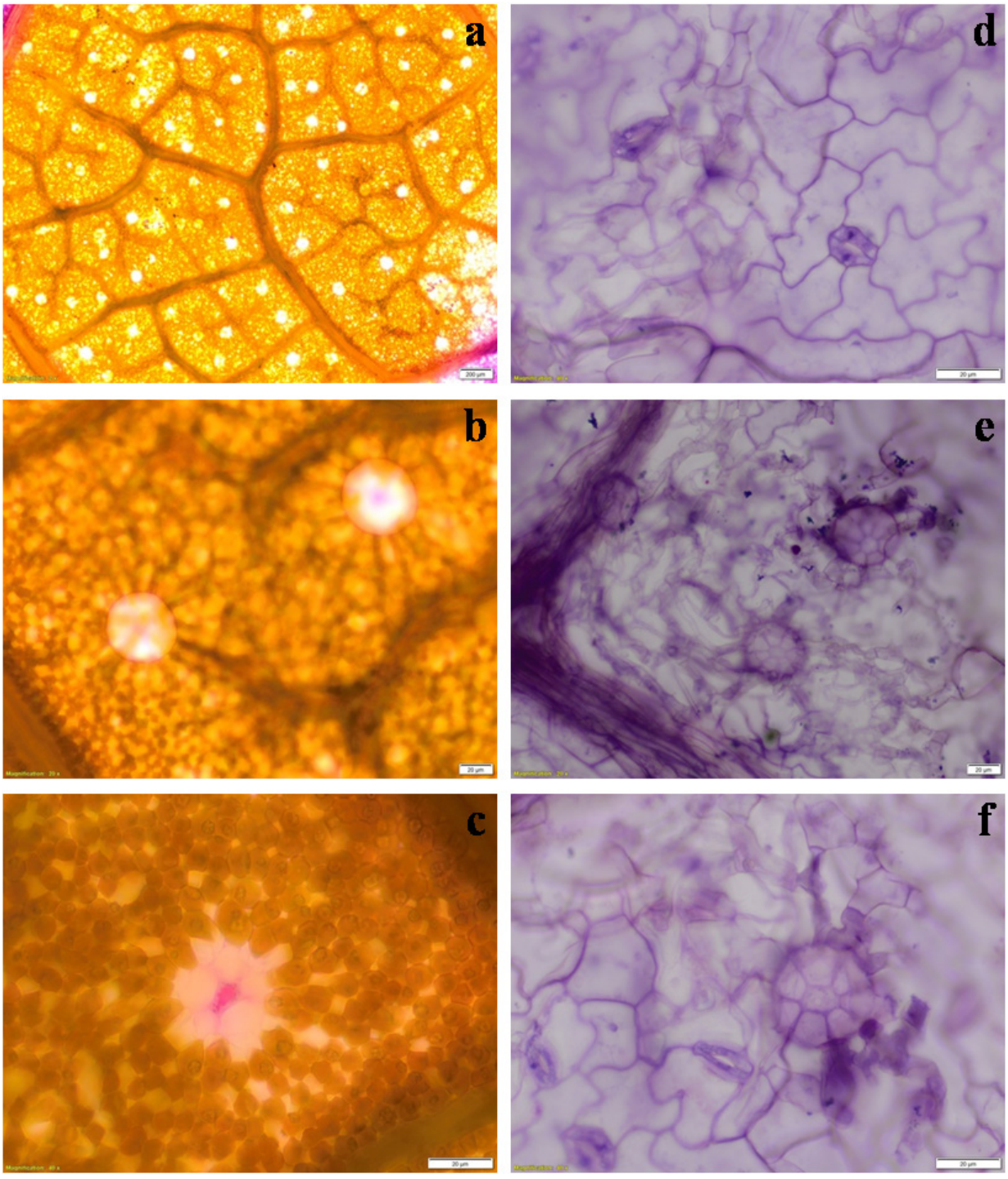

Fig. 3. *Aristolochia ringens* Vahl.: **a**. Distribution of cork warts on the upper epidermis; **b**. Cork-warts associated with idioblast in upper epidermis; **c**. Origin of cork-warts from the disintegration cells surrounding d-type stomata in upper epidermal cells; **d**. Presence of anomocytic stomata, and undulated U-shaped anticlinal walls on the lower epidermis; **e**. Structure and development of cork-warts associated with idioblasts in lower epidermis; **f**. Origin of cork-warts from disintegration cells surrounding d-type stomata in lower epidermal cells.

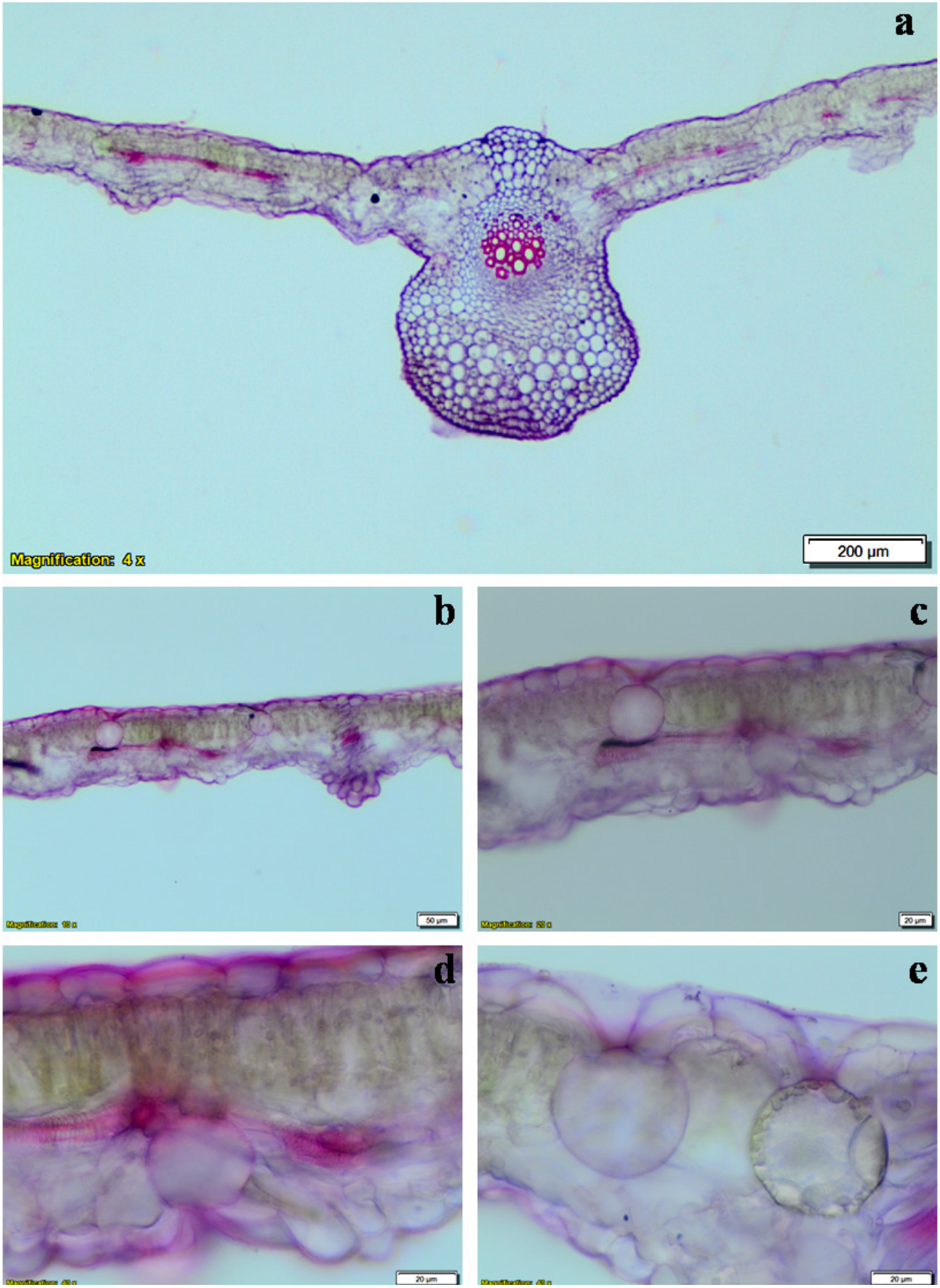

Fig. 4. *Aristolochia ringens* Vahl.: **a.** Cellular organization of mesophyll tissues in midrib and lamina region; **b.** Palisade tissue in single layer, convex and rectanular shaped upper epidermal cells and sparsely arranged spongy tissue; **c.** Cork-wart associated with idioblast below upper epidermis; **d.** Cork-wart associated with idioblast below lower epidermis; **e.** Presence of idioblast with or without oil drops/mucilage like substances.

(ii) Anatomical Analysis

Leaf lamina through the midrib

Leaf lamina have single layered upper and lower epidermis containing elliptical to sub-rectangular/squarish cells covered with thick cuticle in both the species. Upper epidermal cells are comparatively slightly larger than lower epidermal cells in both the species (Fig. 2c &4b). The upper epidermis is followed by two-layered upright cells of palisade tissues and the lower epidermis is followed by loosely arranged 3-4 layers of spongy tissues in *A. indica* (Fig. 2b). A few unicellular multiseriate trichomes are present on upper epidermis while hook like numerous trichomes are present on lower epidermis in *A. indica* (Fig. 2e). In *A. ringens*, the palisade tissue as well as in spongy tissue is interrupted by idioblastsat some places just below the upper and lower epidermises (Fig. 4c &d). Idioblasts are rounded in shape some contain oil drops and some are empty. The idioblasts are opened on the upper and lower epidermis respectively by a tiny hole opening called cork-warts. They are present on both surfaces in *A. ringens* are similar in structure and function whereas upper and lower cork-warts differ in structure in *A. indica*. Upper surface cork-warts lack idioblast associated with it (Fig. 2d) unlike cork-warts found at lower surface (Fig. 2b). Upper and lower epidermal cells are smaller and rounded in shape in the midrib region. 3 to 5 layers of Collenchyma tissues are present below the upper and lower epidermises at midrib in *A. indica* (Fig. 2a) while 6 to 8 layers of collenchyma at the upper epidermis and 2 to 3 layers at the lower epidermis are present in *A. ringens* (Fig. 4a). Vascular bundles are crescent-shaped, xylem arranged in a radial row and phloem lies under the xylem in *A. indica* whereas circular vascular bundles are present in *A. ringens*. Ground tissue is composed oflarge-sized thin-walled rounded parenchyma cells between collenchyma and vascular bundles.

Discussion

Cork-warts are regions covered with suberin, and layers of adjacent rectangular cells that grow from tangential divisions and create circular structures (Joffily & Vieira, 2010). Leaf cork-warts, as described by Stace (1965), are holes encircled by relatively tiny and irregularly shaped epidermal cells as compared to the rest of the normal cells. Baas (1975) discussed the practicality of diagnosing cork-warts using this structure. Poulsen (1875) referred to cork-warts structures as suberized formations (korkdannelse), Bachmann (1880) called them "foliar lenticels," Borzi (1886) and Ross (1896) called them formations, Motte (1926) called them suberized nodules, and Dickison (2000) and Haberlandt (1928) claimed they were suberized areas. According to Morretes and Venturelli (1985), the term "lenticel" is inadequate for cork-warts since there is no phellogen, no intercellular gaps in the complimentary tissues and closing layers, and no suberized and lignified stomata that have ruptured. First used about species of the genus *Ilex*, the word "cork-wart" appeared in the translated version of the book. Further research on the foliar epidermis of other families utilized the name "cork-wart" by Metcalfe and Chalk (1950), Stace (1965), Den Hartog and Baas (1978), and Farooqui (1982).

Specifically, leaf anatomy may aid in systematic research and is crucial for family-level identification (Solereder 1908; Metcalfe & Chalk 1950, 1979; Scotland *et al.*, 2003). In about twenty-one families, Metcalfe and Chalk (1950, 1979) identified cork-warts as a taxonomic indicator. Though cork-warts on leaves have been documented in other plant families, the Aristolochiaceae have yet to be one of them.

While cork-warts caused by damage, fungus, or insects do not have taxonomic significance, cork-warts generated by the breakdown of ordinary epidermal cells, the loss of trichomes, glands in the epidermis, or stomata have much taxonomic value. The current research demonstrated that

cork-warts in *A. indica* and *A. ringens* do not form from insect assault or damage, but rather they are driven by the disintegration of regular epidermal cells and d-type stomata. The presence, structure, and origin of other species of *Aristolochia* and related genera of the family *Aristolochia*ceae could be confirmed through further studies with wider sampling. This would help with taxonomic delimitation within the family and facilitate easier species identification.

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Literature Cited

BAAS P. 1975. Vegetative anatomy and the affinities of Aquifoliaceae, *Sphenostemon*, *Phelline* and *Oncotheca*. *Blumea* 22: 311–407.

BACHMANN E. 1880. Uber Korkwucherungen auf Blattern. Pringsheims Jahrb. J*ahrbücher für Wissenschaftliche Botanik* 12: 190–236.

BORAH D., TARAM M., DAS A.P., TANGJANG S. & T. VAN DO 2019. *Aristolochia assamica* (*Aristolochia*ceae), a new species from the East Himalayas. *Annales Botanici Fennici* 56(4–6): 253–257. https://doi.org/10.5735/085.056.0410

BORZI A. 1886. Di Alcune Lenticelle Fogliari. First Edition. Malpighia, Messina.

BUIJSEN J.R.M. 1995. Leaf anatomy of *Harpullia*, *Majidea* and *Conchopetalum* (Sapindaceae). *Blumea* 40: 345–361.

DEN HARTOG R.M. & P. BAAS 1978. Epidermal characters of the Celastraceae *sensu lato*. *Acta botanica neerlandica* 27(5/6): 355–388.

DICKISON W.C. 2000. *Integrative plant anatomy*. Academic Press.

DUKE N.C. & J.S. BUNT 1979. The genus *Rhizophora* (Rhizophoraceae) in north-eastern Australia. *Australian Journal of Botany* 27: 657–678.

EVANS L.S. & A. BROMBERG 2010. Characterization of cork warts and aerenchyma in leaves of Rhizophora mangle and Rhizophora racemosa. *The Journal of the Torrey Botanical Society* 137(1): 30–38.

FAROOQUI P. 1982. Cork-warts in *Eucalyptus* species. *Proceedings: Plant Sciences* 91: 289–295.HABERLANDT G. 1928. *Physiological Plant Anatomy*. Macmillan Publishers, London.

JOFFILY A. & R.C. VIEIRA 2010. Cork-warts on the leaf epidermis of four genera of Celastroidea-Celastraceae. *Flora* 205: 313–318. https://doi.org/10.1016/j.flora.2009.12.014

KELLER R. 1890. Ueber Erscheinungen des normalen Haarverlustes an Vegetationsorganen der Gefasspflanzen. *Nova acta Academiae Caesareae Leopoldino-Carolinae* 55: 305–360.

KORN R.W. & G.W. FREDRICK 1973. Development of D-type Stomata in the Leaves of *Ilex crenata* var. *convexa*. *Annals of Botany* 37: 647–656.

MATTEUCCI E. 1897. Contributo allo studio delle placche sugherose nelle piante. *Un nuovo giorno in Italia* 4: 224–244.

METCALFE C.R. & L. CHALK 1950. *Anatomy of the Dicotyledons.* First Edition. Clarendon Press, Oxford.

METCALFE C.R. & L. CHALK 1979. Anatomy of the dicotyledons. Second Edition. Claredon Press, Oxford.

MORRETES B.L. & M. VENTURELLI 1985. Ocorrência de "lenticelas" em folhas de *Tripodanthus acutifolius* (R. & P.) Tiegh. (Loranthaceae). *Revista Brasileira de Botânica* 8: 157–162.

MOTTE J. 1926. Sur les nodules subéreux des feuilles d'Eucalyplus globulus. *Bulletin de la Société Botanique de France* 73(5): 1028-1032.

NEINHUIS C., WANKE S., HILU K.W., MULLER K. & T. BORSCH 2005. Phylogeny of *Aristolochia*ceae based on parsimony, liklihood and Bayesian analyses of *trn*L-*trn*F sequences. *Plant Systematics and Evolution* 250: 7–26.

PAGODA I.O., PAUTOV A.A., ZELENSKAYA M.S. & D. YU VLASOV 2015. Cork warts on leaves of *Gnetum* L. (Gnetaceae) and its Phylloplane Fungi. *International Journal of Botany Studies* 11: 10–20. https://doi.org/10.3923/ijb.2015.10.20

POULSEN V.A. 1875. Om Korkdannelse paa Blade. Vid. Med. *Dansk Nat. For. Kjøbenhavn* 37: 44–58.

RASCHE L. & J. KOVAR-EDER 2009. Woody angiosperm taxa of the Canary laurel forests: leaf morphology and cuticular structures. *Stuttgarter Beitr Naturk* 2: 447–494.

RAVIKUMAR K., TIWARI U. & N. BALACHANDRAN 2014. *Aristolochia gurinderii* (*Aristolochia*ceae): A new species from Great Nicobar Island, India. *Phytotaxa* 172: 117–122. https://doi.org/10.11646/phytotaxa.172.2.77

ROSS H. 1896. Sui Ranunculus parviflorus L. e R. Chius DC. della Sicilia. Naturalista Siciliano. *N. Ser. An.,* 1: 107-110.

SMITH F. & E.C. SMITH 1942. Anatomy of the inferior ovary of *Darbya*. *American Journal of Botany* 29: 464–471.

SOLEREDER H. 1908. Systematic anatomy of the Dicotyledons. Claredon Press, Oxford.

STACE C.A. 1965. Cuticular studies as an aid to plant taxonomy. Bulletin of the British Museum (Natural History) Botany 4: 3 –78.

STACE C.A. 1966. The use of epidermal characters in phylogenetic considerations. *New Phytologist* 65: 304–318.

SUDHAKARAN M.V. 2016. Finger Printing of the Anatomical Markers, HPTLC Profile and Heavy Metals Content in the Leaves of *Aristolochia indica* Linn. *Pharmacognosy Journal* 8(2)

VAZ P.P., ALVES F.M., & R.C.O. ARRUDA 2018. Systematic implications of leaf anatomy in the Neotropical *Mezilaurus* group (Lauraceae). *Botanical Journal of Linnean Society* 189 (2): 186-200.