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Article

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Towards a natural classification of Dothideomycetes: clarification of *Aldona*, *Aldonata* and *Viegasella* (*Parmulariaceae*)

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Abstract

Species of *Parmulariaceae* are biotrophic, plant-parasitic microfungi that develop on the surface of living plants. We collected *Aldona stella-nigra* during a survey of foliar epiphytes in the Philippines and thus we restudied this poorly reported species around the world and re-examined some similar taxa. In this paper we re-describe and illustrate the type species of some similar genera, *Aldona*, *Aldonata* and *Viegasella* in *Parmulariaceae* which are leaf parasites and also provide details of the spermatial state of these unusual fungi. By re-illustrating the genera we anticipate fresh collections of these genera to be obtained for further studies so that molecular data can be analyzed to obtain a natural classification.

Key words – Foliar epiphytes – *Parmulariaceae* – types

Introduction

We are studying foliar epiphytes, which are groups of relatively poorly known fungi in Dothideomycetes, Sordariomycetes and Eurotiomycetes, mostly occurring in tropical regions (Spegazzini 1888, Batista & Ciferri 1962, 1963, Dennis 1970, von Arx & Müller 1975, Sivanesan & Sinha 1989, Hyde 1996, 2001, Reynolds & Gilbert 2005, Chomnunti *et al*. 2011, 2012a, b, 2014, Wu *et al*. 2011, Hosagoudar 2012, Hyde *et al*. 2013, Hongsanan *et al*. 2014a, b, c, 2015, Liu *et al*. 2015, Ariyawansa *et al*. 2015). They can be grouped in four or more distinct types based on their appearance on the host and their biology.

The sooty moulds (e.g. *Antennulariaceae*, *Capnodiaceae*, *Chaetothyriaceae*, *Euantennariaceae*, *Meliolinaceae*, *Trichomeriaceae*, *Triptosporiaceae*) form thick blackened mycelial layers on the surface of healthy green leaves and branches of trees, or even grasses, soil and rocks beneath trees, often with several species coexisting together (Chomnunti *et al*. 2011, 2012a, b, 2014, Hyde *et al*. 2013). They grow on the sugary exudates excreted on to the leaves by plant sap sucking insects and although they do not directly damage the host they can reduce yields by reducing photosynthesis (Chomnunti *et al*. 2011, 2014). They may also cause dirty black blemishes on fruits, thus reducing marketability. Their occurrence may become more commonplace on fruit with global warming and the movement towards organic products (Stover 1975).

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The black moulds (*Asterinaceae, Microthyriaceae* and *Meliolaceae*) are generally biotrophic and produce sparse, superficial, web-like arrangements of blackened mycelium on the leaf surface, usually producing appressoria *sensu*, while ascomata or pycnidia develop in the center of the "webs" beneath or above the mycelia (Hosagoudar & Ruji 2011, Wu *et al*. 2011, Hongsanan *et al*. 2014a, c, 2015, Guatimosim *et al*. 2015, Firmino *et al*. 2016). The appressoria form an apoplastic interaction apparatus which form a small "peg" or hyphalic tubes penetrating the cuticle of host, the hyphae with lateral appressoria come from intimal infection and form a dark, web-like colony superficially (Hansford 1946, Hughes 1993, Mibey & Hawksworth 1995, Dean 1997, Gregory & John 1999, Inácio & Cannon 2008, Yi & Valent 2013, Hongsanan *et al*. 2014a, c, 2015, Guatimosim *et al*. 2015). These fungi, however rarely cause significant damage to the host, although they may reduce marketability of fruits or leaves (Hofmann 2010). Again it is likely their occurrence on fruit will become more commonplace with the occurrence as global warming and the movement towards organic products.

A third group named fly speck fungi, often cause blemishes on fruits which are mostly caused by genera of *Schizothyriaceae*. They form clusters as ascomata on fruits linked by superficial mycelia (Batzer *et al*. 2008, Ivanović *et al*. 2010, Gleason *et al*. 2011, Phookamsak *et al*. 2016). Again these fungi, rarely cause primary damage to the host, however they may cause the economic losses. The fourth group, which is perhaps a subgroup of group 2, are inconspicuous and often only visible as single or groups of fruiting bodies, often on the lower surface of leaves of tropical trees. These secretive fungi appear to produce structures under the fruiting bodies that penetrate host cells and obtain nutrients, although in some cases they may cause leaf necrosis and probably feed on dead plant cells. The genera of *Aulographaceae, Micropeltidaceae* and *Parmulariaceae* make up this group (Inácio & Cannon 2008, Hongsanan *et al*. 2014a, b, c, Guatimosim *et al*. 2015).

Most of foliar epiphytes are relatively poorly known and have rarely been subjected to sequence analysis (Wu *et al*. 2011, Hongsanan *et al*. 2014a, b, c, 2015, Wijayawardene *et al*. 2014). This is because it is impossible to isolate these biotrophs into culture or in case of sooty moulds because they comprise several species intermingled in a sooty mould colony (Chomnunti *et al*. 2014).

We are studying the foliar epiphytes in order to understand their role, as well as their phylogenetic relationships with other fungi (Chomnunti *et al*. 2011, 2012a, b, 2014, Wu *et al*. 2011, 2014a, b, Hongsanan *et al*. 2014a, c, 2015). Most species are Ascomycota, but it may be possible that some are Basidiomycota. These fungi may also be important as it is likely they produce unique novel chemicals as they occupy unusual niches, belong to chemically poorly studied genera and are members of poorly known families (Bills 1996, Aly *et al*. 2011, Debbab *et al*. 2011, 2012, 2013). We collected a species of *Aldona* during a survey of foliar epiphytes in the Philippines which matches *A. stellata-nigra* Racib. In this paper, we compared *Aldona* with some morphologically similar taxa, *Aldonata* and *Viegasella* in *Parmulariaceae*. We also provided details of the spermatial states of these unusual fungi.

Materials & methods

Morphological study

Fresh specimens were collected from the Philippines, while type specimens were obtained from M, K and S (for full names of herbaria see http://sweetgum.nybg.org/ih/index.php). One or two fruiting bodies were rehydrated in 3%-5% KOH and transferred by fine forceps to a drop of water on a slide. Microscope slide mount were prepared with water and lactophenol with Cotton blue reagent. Sections of ascomata were made by free hand and mounted in lactic acid. Observations and hand sections were examined under a stereoscope (Nikon ECLIPSE 80i) and photographed by a Canon 550D digital camera fitted to the microscope. Measurements were made with Tarosoft (R) Image Frame Work and photographic plates processed and improved using Adobe Photoshop CS5 (Adobe Systems Inc., The United States).

Results

Although we obtained a culture of our fresh collection of *Aldona stella-nigra* from single ascospores and sequenced this (ITS, SSU), a blast search showed it to be close to *Meira argovae* (GenBank No. AY15867). *Meira argovae* is accommodated in Exobasidiomycetes which causes leaf and flower gall disease (Basidiomycota, *Incertae sedis* order and family level). The two taxa cannot be confused as *A. stella-nigra* is an ascomycete with asci and ascospores, while *M. argovae* belongs in Exobasidiomycetes with sori in leaves which produce basidiospores. We suspect that a basidiomycetous yeast was growing in association with the ascomycete and outgrew our fungus in culture.

Taxonomy

Aldona Racib., Parasit. Alg. Pilze Java's (Jakarta) 1: 19 (1900)
Index Fungorum number: IF 115
Facesoffungi number: FOF00309

Parasitic growing on the surface of living leaves. *Leaf spots* amphigenous and less visible at upper leaf surface, solitary to gregarious, orbiculare, brown, black in center, yellowish swollen on leaf surface, different in appearance between leaf upper and lower sides. *Ascomata* gregarious, semi-immersed to erumpent, black, linear, radial or star-shaped, branching, coriaceous, shiny, opening by longitudinal slits. *Wall of ascoma* composed of amorphous black tissue, base thin composed of brown cells of *textura angularis. Hamathecium* of dark brown to hyaline, branching, pseudoparaphyses. *Asci* 8-spored, bitunicate, clavate to cylindrical, with a relatively long pedicel, apically rounded with an ocular chamber. *Ascospores* 2-7- septate, elongate-clavate, upper cells larger and wider, basal cells short and narrow, hyaline, trans-septate, constricted at the septa, especially between the second and third cells from the apex, wall smooth. *Spermatogonia* solitary to gregarious, black, shiny, globose or irregular, mostly growing on the spot, surrounded by ascomata. *Wall of Spermatogonia* comprising 2 layers, outer layer thick and composed of darkly pigmented cells, inner layer composed of hyaline to pale brown cells of *textura angularis*. *Spermatophores* reduced to spermatogenous cells. *Spermatogenous cells* hyaline, enteroblastic, phialidic, determinate, forming from the inner cell walls, broadly at the basal and narrow at apex. *Spermatia* aseptate, fusiform, hyaline or sometime slightly greenish, tapering at both ends, smooth-walled, with a gelatinous matrix.

Notes: *Aldona* was described by Raciborski (1900) as parasite on living leaves of *Pterocarpus indicus* Willd. and placed in *Hysteriaceae* (Saccardo 1904; Penzig & Saccardo 1904). It was transferred to *Phacidiaceae* by Von Höhnel (1917). Bisby (1923) and Zogg (1962) also suggested that *Aldona* cannot be placed in *Hysteriaceae*. *Aldona* however has bitunicate asci, but species in *Phacidiaceae* have unitunicate asci and therefore Nannfeldt (1932) suggested this genus cannot be accommodated in *Phacidiaceae*. Teodoro (1937) also listed this genus from living leaves of *Pterocarpus* sp. in the Philippines. Müller & Patil (1973) provided a key to the species based on the distinction of ascomatal shape and ascospore form, and they referred to *Aldona* with three species: *A. stella-nigra* Racib., *A. americana* Petr. & Cif. and *A. minima* E. Müller & B.V. Patil. All species are from living leaves of *Pterocarpus* species. Sivanesan & Sinha (1989) introduced a new genus *Aldonata* which is morphologically distinct in the ascospore septation and septum ontogeny with *Aldona*. Therefore, consideration of both genera indicated that there are differences based on ascospores and ascomata (*Aldonata* has much larger locules and muriform rather than transversely septate ascospores), we accept both of them as different genera in *Parmulariaceae*. At present the genus *Aldona* is placed in *Parmulariaceae* with three species (Inácio & Cannon 2008, Hyde *et al*. 2013).

Aldona stella-nigra Racib, Parasit. Alg. Pilze Java's (Jakarta) 1: 19 (1900) Figs 1–3
Index Fungorum number: IF 172102
Facesoffungi number: FOF00310

Key to genera discussed in this paper

1. Ascomata superficial……...*Viegasella*
1. Ascomata initially immersed and then becoming erumpent or superficial……2
2. Ascospores with only transverse septa……*Aldona*
2. Ascospores with transverse and longitudinal septa……...*Aldonata*

Parasitic growing on the upper and lower surface of living leaves. *Leaf spots* amphigenous and less visible at upper leaf surface, solitary to gregarious, orbiculare, brown, black in center, yellowish swollen on leaf surface, different in appearance between leaf upper and lower sides. Spot on upper leaf less distinct, 3–10 mm diam, reddish brown to light brown; Spot on lower surface more distinct, larger, 4–12 mm diam, cream to fawn. *Ascomata* 1–3 mm long × 0.2–0.4 mm wide ($\bar{x}$ = 1.8 × 0.32 μm, n = 5), semi-immersed to erumpent, appearing as flexuous lines, branching with other ascomata and form a black star in the middle of the leaf spot, opening by longitudinal slits, hard to remove from leaf surface, gel-like or soft when wet, brittle when dry. *In vertical section,* 230–615 μm deep, comprising separate blackened upper and lower walls. *Upper wall* 2-layered, 45–92 μm thick, brittle, outer layer composed of brown thick-walled cells of *textura angularis*, inner layer comprising colorless thin-walled cells; *Lower wall* 1-layered, 9–17 μm thick, comprising poorly defined, dark brown to black cells of *textura angularis*, with plant tissue. *Hamathecium* 0.6–1.4 μm diam, filamentous pseudoparaphyses, anastomosing in gelatinous matrix with asci embedded in mucilage. *Asci* 59–84 μm long × 14–31 μm wide ($\bar{x}$ = 68 × 21 μm, n = 10), 8-spored, bitunicate or fissitunicate, broadly fusiform to obovoid, thick at apex, short and narrow pedicellate or occasionally with long and narrow pedicellate, with an ocular chamber, forming from the base of the ascomata, asci vertically arranged and embedded in a gelatinous matrix, not changing color in IKI. *Ascospores* 28–48 μm long × 7–10 μm wide ($\bar{x}$ = 37 × 8 μm, n = 10), 2-3-seriate, long clavate, hyaline, 2-7- septate, mostly 5-septate, constricted at each septum; ascospore with more than 5 septa has the deepest constriction at the upper second septum, apical cells wider and shorter, basal cells longer and narrower, both ends rounded, smooth-walled. *Spermatogonia* 120–335 μm long × 75**–**205 μm wide ($\bar{x}$ = 264 × 154 μm, n = 10), solitary to gregarious, black, shiny, carbonaceous, globose to subglobose or irregular, mostly growing on the spot, surrounded by ascomata, hard to remove from leaf surface. *Wall of Spermatogonia* 25–45 μm thick ($\bar{x}$ = 33 μm, n = 7), comprising 2 layers, outer layer thick and composed of darkly pigmented 15–35 μm diam ($\bar{x}$ = 23 μm, n = 7) cells, inner layer composed of hyaline to pale brown 10–20 μm diam ($\bar{x}$ = 15 μm, n = 7) cells of *textura angularis*. *Spermatophores* reduced to spermatogenous cells. *Spermatogenous cells* 15–20 μm long × 0.5–2 μm wide ($\bar{x}$ = 17 × 1 μm, n = 10), hyaline, enteroblastic, phialidic, determinate, forming from the inner cell walls, embedded in a gelatinous matrix with spermatia groups, broadly at the basal and narrow at apex. *Spermatia* 3–3.5 long × 1–1.5 μm wide ($\bar{x}$ = 1 × 3 μm, n = 10), aseptate, fusiform, narrow at both ends broad at the center, hyaline or sometime slightly greenish, smooth-walled, with a gelatinous matrix.

Material examined **–** INDONESIA, Sumatra, on leaves of *Pterocarpus indicus* Willd (*Fabaceae*), 20 February 1959, Raciborski (M! 176025, **isotype**).

Other specimen examined **–** PHILIPPINES, Los Baños: Mt Makiling, on living leaves of *Pterocarpus draco* L. (*Fabaceae*), February 2012, K.D. Hyde (MFLU14-0011).

Aldonata Sivan. & A.R.P. Sinha, Mycol. Res. 92(2): 248 (1989)

Index Fungorum number: IF 25242

Facesoffungi number: FOF00311

Parasitic on leaf spots. *Spots* solitary, scattered, sometimes confluent, variable in shape, cicular to irregular, greyish-white, edge diffuse, amphigenous. *Ascomata* semi-immersed, globose to subglobose, black, shiny, appearing as flexuous lines on the leaf spot surface with a clearly-defined margin, hard remove from leaves surface. *Wall of ascoma* 1-layered, composed of poorly-defined brown to black cells. *Hamathecium* transverse septate, long, colourless, branched, pseudoparaphyses. *Asci* 8-spored, bitunicate, clavate to broadly clavate, pedicellate, thin-walled.

Fig. 1 – *Aldona stella-nigra* (M! 176025, isotype). a Spots on lower surface of leaves. b–d Black ascomata with longitudinal openings. e, f Section of ascomata. g–i Clavate to cylindrical asci containing eight ascospores. j–l Hyaline ascospores with 4-7 septa. – Bars: a = 50 mm, b–d = 1 mm, e, f = 100 µm, g–l = 10 µm.

Ascospores multiseriate, ellipsoid to fusiform, muriform, hyaline, with up to 8 transverse and longitudinal septa, lower cell narrow and longer, caudiform, with a mucilaginous sheath. *Spermatogonia* solitary to gregarious, sub-immersed, black and shiny, carbonaceous, globose to irregular, mostly growing around the central of grayish-white spot. *Wall of Spermatogonia* 1-layered, composed of thick-walled colorless cells of *textura angularis*. *Spermatophores* reduced to Spermatogenous cells. *Spermatogenous cells* hyaline, enteroblastic, phialidic, determinate, arising from basal cells of inner peridium wall. *Spermatia* aseptate, fusiform to cylindrical, hyaline, tapering at both ends, smooth-walled.

Notes: *Aldonata* was introduced by Sivanesan & Sinha (1989) as a monotypic genus, it contains the single species *Aldonata pterocarpi* Sivan. & A.R.P. Sinha. The genus is characterized by much larger locules than *Aldona* and muriform ascospores. *Aldonata* is presently placed in *Parmulariaceae* (Inácio & Cannon 2008), and is similar to *Aldona*. *Aldona* and *Aldonata* are only genera of *Parmulariaceae* which have colourless ascospores and are occur on legumes (Inácio & Cannon 2008). *A. pterocarpi* was described and illustrated in Inácio & Minter (2002) which has the grayish-white lines surrounding the colony and are similar to the cream-coloured lines in *Aldona stella-nigra* (Inácio & Minter 2002). The asexual state of *Aldonata pterocarpi* has not previously been reported, but we found pycnidia surrounding the ascomata.

Fig. 2 – *Aldona stella-nigra* (MFLU14-0011). a, b Colony on lower side of living leaves. c, d Ascomata on yellowish leaf spot. e, f Vertical section of ascoma. g Asci arrangement in gelatinous matrix. h Hamathecium. i Ascus tip, note ocular chamber in Melzer's reagent. j–l Asci with ascospores, note k mounted in Melzer's reagent and l mounted in cotton blue reagent. m Germinating ascospore, n–s Ascospores, note the long and narrow ends, q and s mounted in Melzer's reagent. s mounted in cotton blue reagent. – Bars: e, f = 100 μm, g = 50 μm, i = 10 μm, h, j–s = 20 μm.

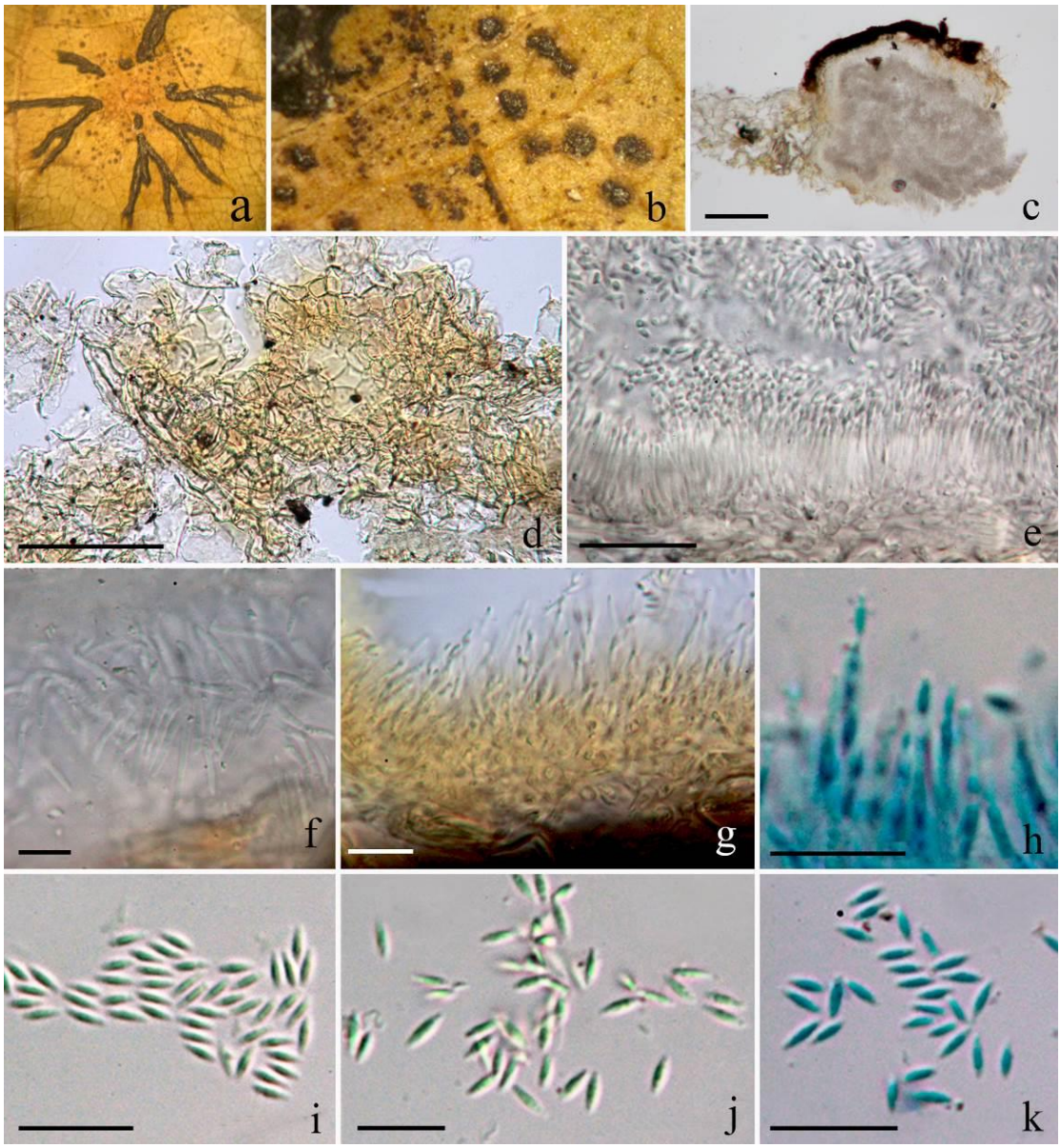

Fig. 3 – *Aldona stella-nigra* (MFLU14-0011). a, b Yellow colony with spermatogonia occurring on lower surface of living leaves. c Section through spermatogonium. d Yellow tissues of spermatogonium. e–g Hyaline spermatia and spermatophores. h Spermatogenous cells. i Aseptate spermatia. j Spermatia in Melzer's reagent. k Spermatia in cotton blue reagent. – Bars: c, d = 100 µm, e = 20 µm, f–k = 10 µm.

Aldonata pterocarpi Sivan. & A.R.P. Sinha, Mycol. Res. 92(2): 249 (1989) Fig. 4.
Index Fungorum number: IF 134615
Facesoffungi number: FOF00312

Parasitic on leaf spots of *Pterocarpus draco*. *Spots* solitary, scattered, sometimes confluent, necrotic in their centers which are often fragile and vrittle, variable in shape, cicular to irregular, greyish-white to light brown, edge diffuse, amphigenous. *Ascomata* 0.8–1.3(–5) × 0.4–0.6 mm ($\bar{x}$ = 1 × 0.5 mm, n = 10), semi-immersed in leaves, globose to subglobose, black, shiny, appearing as flexuous lines on the leaf surface with a clearly defined margin, branching with other ascomata to form a black star in the center of leaf spot, opening by longitudinal split when wet. *Wall of ascoma* 1-layered, apex brittle, 13–51 µm thick, often fragmenting during sectioning, thicker at base and sides, up to 32 µm thick, composed of poorly-defined brown to black cells of *textura angularis*, 2–5 µm diam. *Hamathecium* of 1–2 µm broad with transverse septate, long, colourless, branched pseudoparaphyses. *Asci* 65–95 × 15–30 µm ($\bar{x}$ = 78 × 22 µm, n = 6), 8-spored, bitunicate, clavate

to broadly clavate, pedicellate up to 34 μm long, thin-walled, with ascospores arranged in a cluster. *Ascospores* 30–55 × 8–11 μm ($\bar{x}$ = 41 × 9 μm, n = 10), multiseriate, ellipsoid to fusiform, muriform, hyaline, with up to 8 transverse septa and some longitudinal septa, constricted at the transverse septa, lower cell narrow and longer, caudiform, with a thin mucilaginous sheath. *Spermatogonia* 80–155 × 75–135 μm diam ($\bar{x}$ = 120 × 100 μm, n = 10), solitary to gregarious, sub-immersed, black and shiny, carbonaceous, globose to irregular, mostly growing around the center of grayish-white spot. *Wall of Spermatogonia* up to 12 μm broad, composed of 1-layered of thick-walled colourless cells of *textura angularis*. *Spermatophores* reduced to *Spermatogenous* cells. *Spermatogenous cells* 5–9 × 1–2 μm ($\bar{x}$ = 6 × 1.5 μm, n = 10), hyaline, enteroblastic, phialidic, determinate, arising from basal cells of inner peridium wall. *Spermatia* 2–4 × 1–1.5 μm ($\bar{x}$ = 3 × 1 μm, n = 10), aseptate, fusiform to cylindrical, hyaline, tapering at both ends, smooth-walled.

Material examined – INDIA, Andaman Islands: Port Blair, on leaves of *Pterocarpus draco* L. (*Fabaceae*), 1 November 1987, A. R. P. Sinha (K! IMI 322833, **holotype**).

Viegasella Inácio & P.F. Cannon, Mycol. Res. 107(1): 82 (2003)
Index Fungorum number: IF 28709
Facesoffungi number: FOF00313

Parasitic on the upper leaf surface. *Colonies* solitary, scattered, sometimes confluent, variable in shape, cicular to irregular, light brown to reddish with a diffuse edge. **Sexual morph –** *Ascomata* superficial, black, shiny, appearing as flexuous lines on the leaf surface, opening by longitudinal splits. *Ostiole* conspicuous. *Peridium* thick at the sides, composed of brown to black thick-walled cells of *textura angularis*, outer brown to black carbonaceous substance and internal hyaline cells, two-layered. Upper and lower wall thin, not well developed, sometimes absent. *Hamathecium* filamentous pseudoparaphyses, with transverse septa, long, colourless, branched, and verrucose at the tips. *Asci* 8-spored, bitunicate, cylindrical to clavate, short-pedicellate, thin-walled, embedded in mucilage, not changing color in IKI. *Ascospores* biseriate or multiseriate, ellipsoid to fusiform or ellipsoid to narrowly ovoid, verrucose, usually two-celled, normally unequal, constricted at each transverse septum, upper cell wider, lower cell narrow and longer, hyaline, becoming pale brown when spores are senescent, each cell has an oil drop. **Asexual morph** – Undetermined.

Notes: *Viegasella* was introduced by Inácio & Cannon (2003) to accommodate *Schneepia pulchella* because of its internal and external stromata and haustoria and was placed in the family *Parmulariaceae*. Its superficial similarity to a lichen was mentioned by Spegazzini (1888). *Viegasella* was compared with *Parmularia*, *Symphaeophyma* and *Mintera* by Inácio & Cannon (2003). It is difficult to distinguish *Viegasella pulchella* and *Aldonata pterocarpi* based only on the macroscopic characters such as orientation of ascomata as both species has the grayish-white to reddish lines surrounding the colony. *Viegasella pulchella* has tiny black spots around ascomata at the edge of leaf spots and 1-septate ascospores which differ from *Aldonata pterocarpi* which has the tiny black conidiomata at center of leaf spots surrounded by the ascomata with muriform ascospores. The asexual state of *Viegasella pulchella* has not previously been reported. The pycnidia-like black tiny dots surrounding the ascomata lack contents.

Viegasella pulchella (Speg.) Inácio & P.F. Cannon, Mycol. Res. 107(1): 83 (2003)

Index Fungorum number: IF 373406, *Facesoffungi number*: FOF00314, Fig 5.

≡ *Parmularia pulchella* (Speg.) Sacc. & P. Syd., Syll. fung. (Abellini) 14(2): 709 (1899)

≡ *Schneepia pulchella* Speg., Anal. Soc. cient. argent. 26(1): 55 (1888)

Parasitic on the upper leaf surface. **Sexual morph –** Spots solitary, scattered, sometimes confluent, variable in shape, cicular to irregular, light brown to reddish with a diffuse edge. *Ascomatal locules* 42–98 × 137–338 μm ($\bar{x}$ = 82 × 274 μm, n = 10), superficial, black, shiny, appearing as flexuous lines on the leaf spot surface, opening by longitudinal splits. *Peridium* thick at the sides, 27–34 μm ($\bar{x}$ = 31 μm, n = 10), composed of brown to black thick-walled cells of *textura angularis*, outer cells thick, brown to black, and internal hyaline cells, two-layered. Upper

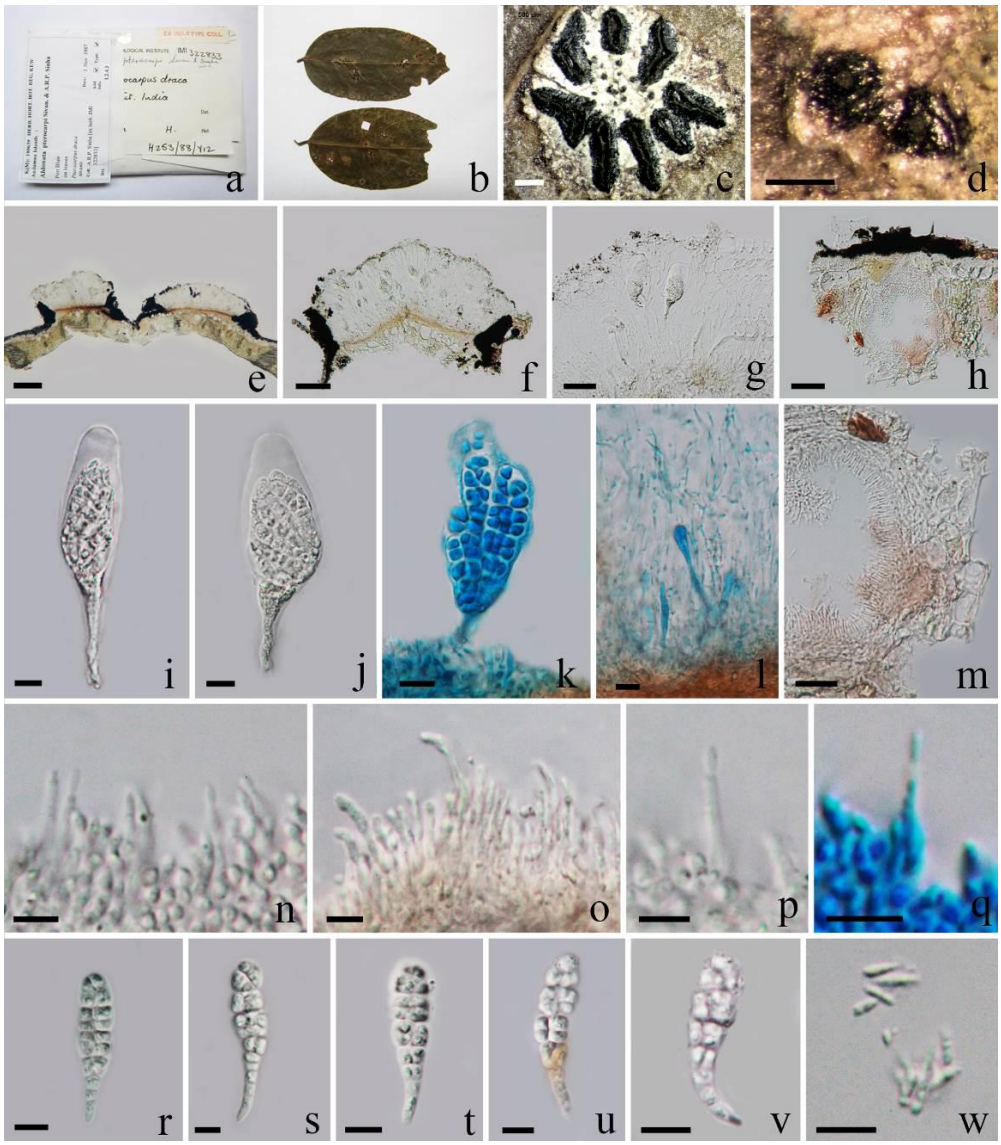

Fig. 4 – *Aldonata pterocarpi* (K! IMI 322833, holotype). a Herbarium labels. b Herbarium material. c Ascomata on host surface. d Spermatogonia on host surface. e–g Section of ascomata. h Section of spermatogonium. i–k Asci with ascospores, note k mounted in cotton blue reagent. l Hamathecium. m Wall of spermatogonium. n–q Spermatogenous cells. r–v Ascospores, note the long and narrow ends. w Spermatia – Bars: c = 500 µm, d, e = 200 µm, f = 100 µm, g, h = 50 µm, m = 20 µm, i–l, r–v = 10 µm, n–q, w = 5 µm.

and lower wall thin, not well developed, sometimes absent. *Hamathecium* 2–5 µm broad with transverse septa, filamentous pseudoparaphyses, long, colourless, branched, and verrucose at the tips. *Young asci* variable in shape, cylindric-clavate to clavate, with a subapical chamber visible before spore delimitation. *Mature Asci* 72–87 × 11–24 µm ($\bar{x}$ = 66 × 16 µm, n = 10), 6-8-spored, bitunicate, cylindrical to clavate, thick-walled particularly in the upper part, short-pedicellate, thin-walled, embedded in mucilage, not changing color in IKI. *Ascospores* 11–20 × 6–8 µm ($\bar{x}$ = 16 × 6 µm, n = 10), uniseriate or biseriate, ellipsoid to fusiform or narrowly ovoid, verrucose, usually two-celled, normally unequal, upper cell wider, lower cell narrow and longer, transverse septa obviously shrink, hyaline and then becoming light brown when spores are senescent, with an oil drop, the apex rounded and the base obtuse, the mucilaginous sheath degenerating at maturity. **Asexual morph** – Undetermined.

Material examined – PARAGUAY, Guarapi, on leaves of *Sapotaceae* sp., November 1883, B. Balansa (PL. Paraguay exs. 4084, K (M): 180636, **isotype**).

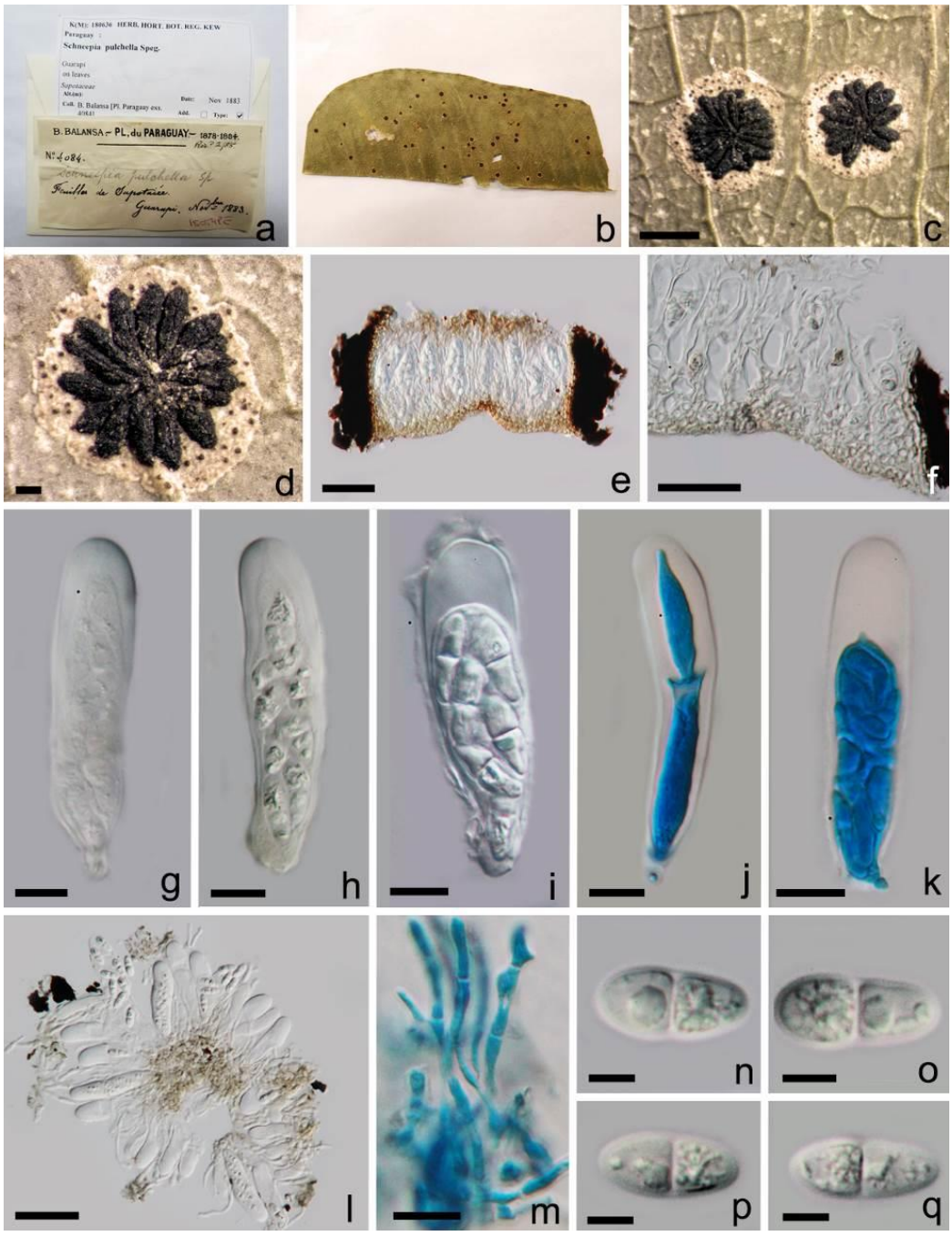

Fig. 5 – *Viegasella pulchella* (K (M): 180636, isotype). a Herbarium labels. b. Herbarium material. c, d Ascomata on host surface. e, f Section of ascomata. g–l Asci with ascospores, note j and k mounted in cotton blue reagent. m Hamathecium. n–q Ascospores. – Bars: c = 1000 µm, d = 100 µm, e, f, l = 50 µm, g–k, m = 10 µm, n–q = 5 µm.

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