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Standard Paper

Lichen symbiont interfaces revisited: ultrastructure of intraparietal contacts between fungal and algal cells in several microlichens with non-trebouxialean chlorobionts

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Abstract

This work attempts to better understand the significance of morphological diversity among fungal-algal contact zones present in lichens. We used TEM to examine a variety of lichen symbioses involving non-trebouxialean green algae that show intraparietal penetration by the mycobiont. A principal focus was on *Endocarpon pusillum*, a well-known member of a family (*Verrucariaceae*; *Eurotiomycetes*) previously reported to be characterized by unwalled haustoria exposing a naked fungal protoplast. Peg-like haustoria arose from an inner layer(s) of the mycobiont cell wall that broke through outer layers and penetrated a short distance into the wall of the green algal symbiont (*Diplosphaera*). In both fungal and algal cells at the contact interface, lomasome-like vesicles and tubules occurred as modifications of the plasmalemma intermixed with wall materials at the inner surface of the cell wall. A fungal cell wall was consistently present around the haustorium, which resembled those depicted in earlier TEM studies of *Verrucariaceae.* Previously published micrographs of *Verrucariaceae* purporting to show wall-less haustoria surrounded by an empty space are believed to have been misinterpreted. However, in the isidiose *Porina* and foliicolous *Calopadia*, *Byssoloma* and *Fellhanera* species (*Lecanoromycetes*), we did observe extreme degrees of reduction in the mycobiont cell wall at symbiont contact interfaces. In those lichens, a broad area of the fungal cell bulged into the adjacent algal symbiont, broadly invaginating the wall of the latter and penetrating it intraparietally without differentiation of a distinct haustorial structure. The mycobiont wall surrounding such protrusions often thinned to near indistinguishability towards its extremity. The protrusion made direct contact with the algal cell wall; no empty space occurred between them. We propose that the short, peg-like intraparietal haustoria bind the symbionts and help maintain cell contacts amid the stresses of tissue expansion and shrinkage, thereby avoiding disruption of the continuous hydrophobic coating that facilitates transfer between them. Broader contact interfaces with extremely thin adjacent walls may facilitate solute flow between symbionts. Reciprocal penetration of algal protrusions into mycobiont cells, noted in *Porina* as well as other lichens studied previously, is a neglected but potentially significant indication that both symbionts may actively work to maintain functional contact interfaces.

Keywords: cell wall; haustoria; lomasomes; photobiont; phycobiont; plasmalemmasomes; symbiosis

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Introduction

The lichen symbiosis is a biologically intimate collaboration that has arisen many times independently within different clades of higher fungi (Lücking *et al.* 2017) in combination with diverse lineages of algal partners (Sanders & Masumoto 2021). Since the relationship was first recognized (Schwendener 1868, 1869), substantial interest has centred on the zones of contact between fungal and algal cells. It was evident that in lichens a fungus exploits a photosynthetic organism as a food source, a situation for which many other examples were already well known (de Bary 1866). Based on comparisons with plant pathogenic fungi, mycobiont penetrations into algal cells were identified as haustoria. Initially, their presence was thought to be exceptional; few lichens appeared to have them (Bornet 1873; Paulson & Hastings 1920; Nienburg 1926). However, careful observations and more thorough surveys (Tschermak 1941; Plessl 1963) eventually revealed that fungal penetration into the algal cell lumen (intracellular) was quite common in simple crustose lichens lacking organized tissues, while in more complex forms with differentiated fungal and algal layers, the mycobiont frequently bored into the algal cell wall without fully traversing it (intraparietal) or made superficial contact only (appositional). The observations and line drawings made by Tschermak (1941) and Plessl (1963), near the limits of the light microscope's resolution, were corroborated in nearly all essential details by subsequent transmission electron microscope studies (e.g. Moore & McAlear 1960; Chervin *et al.* 1968; Peveling 1968; Galun *et al.* 1970, 1971*a*; Honegger 1984, 1986*a*; Matthews *et al.* 1989). However, the significance of the penetrative contacts,

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which so often do not even enter the algal cell lumen, has remained enigmatic.

Although 'haustorium' is usually defined as a specialized hyphal branch that serves to absorb food (Kirk *et al.* 2001), in lichens the term is routinely applied to penetrative structures that are unlikely to play a central role in obtaining nutrition. Physiological studies of substance transfer in lichens suggest mass leakage of carbohydrate by the algal symbionts (Richardson *et al.* 1968; Smith *et al.* 1969; Smith 1980), rather than a directed removal via fungal absorptive structures. Autoradiographic studies confirm that carbohydrate flows across the entire algal wall surface, not merely through the haustorial interface (Jacobs & Ahmadjian 1971; Hessler & Peveling 1978). Applying quantitative arguments, Collins & Farrar (1978) went so far as to conclude that lichen haustoria play no role at all in carbohydrate transfer. Further research revealed a fine layer of mycobiont-secreted hydrophobic materials that coats the exterior wall surfaces of the contacting symbionts, channelling the flow of leaked photosynthate and any other solutes apoplastically between them (Honegger 1986*b*, 1991; Trembley *et al.* 2002). This discovery provided a more lucid understanding of how substances released over the entire cell surface can be passed efficiently between the symbionts. What has remained unclear is whether the morphology of the contact zone (intracellular, intraparietal, or appositional) has any real implications for inter-symbiont transfer, or whether the diversity of those interfaces might be better explained by mechanical factors and/or phylogenetic history.

If the algal symbiont is releasing photosynthate over its entire cell surface, the fungus would seem to have little to gain nutritionally by penetrating into algal cells. On the other hand, the dense fungal and algal cell walls, although theoretically porous, might to some extent slow down the flow of solutes between symbionts (Tschermak-Woess 1988). If that is the case, a reduction in wall thickness could conceivably improve transfer efficiency. Although attention is usually focused on the degree of degradation of the algal cell wall by the fungal haustorium, several authors have also noted substantial thinning of the fungal wall in zones of symbiont cell contact (Chervin *et al.* 1968; Malachowski *et al.* 1980; Tschermak-Woess 1983; Honegger 1984; Büdel & Rhiel 1987). Extreme cases have been reported in TEM studies of *Verrucariaceae* by Galun *et al.* (1971*b*, 1973), Kushnir & Galun (1977), and Galun (1988), who described the complete dissolution of the fungal cell wall surrounding the haustorium, such that a naked fungal protoplast emerged into a space formerly occupied by the dissolved wall materials. However, their published micrographs are not unambiguously supportive of this interpretation. In those figures, the area identified as a 'space' surrounding the putatively naked protoplast is a highly electron-transparent zone that is continuous and indistinguishable from the equally electron-transparent band clearly corresponding to the fungal cell wall elsewhere in the micrographs (fig. 4 in Galun *et al.* (1971*b*); figs 5–7 in Galun *et al.* (1973); fig. 3 in Kushnir & Galun (1977)). Perhaps some clarifying detail was lost in the reproduction of those images, but there is reason to question whether they in fact show the fungal haustorium without its cell wall. Considering the potential implications for interpreting the structure in the context of inter-symbiont transfer, further investigation is needed. In the present work, we examine the symbiont contact zone in *Endocarpon pusillum* Hedw., a well-known member of the *Verrucariaceae* (*Eurotiomycetes*), and compare it with divergent examples from two isidiose *Porina* species (*Gyalectales*; *Lecanoromycetes*) and several foliicolous lichens (*Lecanorales*) showing different interface morphologies and degrees of intraparietal penetration and wall reduction. We attempt to determine whether haustorial walls are absent, reduced, or reinforced in these taxa, and consider the implications for the functional significance of lichen haustoria in their diverse forms.

Methods and Materials

Lichen samples were collected from south-western Florida, USA (*Calopadia puiggarii* (Müll. Arg.) Vězda, *Endocarpon pusillum*, *Porina microcoralloides* Ertz *et al.* and *P. nanoarbuscula* Ertz *et al.*), and Tenerife (*Byssoloma kakouettae* (Sérus.) Lücking & Sérus., *B. leucoblepharum* (Nyl.) Vain. and *Fellhanera bouteillei* (Desm.) Vězda), Spain, in the course of other studies focused on these taxa (Sanders *et al.* 2016, 2023; Sanders & de los Ríos 2017, 2023).

Fresh material, misted in Petri plates with distilled water 24 h previously, was hand-sectioned with a thin razor blade. Sections were placed immediately into ice-chilled tubes with 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.1) for *c.* 3 h, then washed in buffer three times in 3 h, post-fixed with 1% osmium tetroxide (5 h), washed again, then dehydrated in a graded ethanol series (25-35-50-75-95-100(×3)%) followed by propylene oxide. Specimens were next infiltrated with Spurr's low viscosity resin (initially diluted with propylene oxide) for 72 h and polymerized at 60 °C (de los Ríos & Ascaso 2002). Specimen blocks were sectioned with an Ultracut ultramicrotome, stained with uranyl acetate and lead citrate, and imaged with a JEOL JEM 1400 Flash transmission electron microscope.

Results

Endocarpon pusillum *and* Diplosphaera *phycobiont*

Earliest indications of intraparietal penetration appeared as a heterogeneous area of disruption within the algal cell wall that soon acquired electron density (Fig. 1A & B, horizontal arrow). The adjacent fungal cell wall showed discontinuity in the outer layers (Fig. 1A & C, double arrow) while inner wall layers expanded into the gap (Fig. 1A, oblique arrows) and became thickened (Fig. 1C). The fungal cell wall layers, especially the inner ones, were highly electron-transparent. Later stages showed the thickened protrusion of fungal wall material projecting further into the zone of algal wall degradation in front of it (Fig. 1D & E, Fig. 2A). Lomasome-like vesicular and tubular membrane modifications were present in the cytoplasm of both symbionts adjacent to the zone of incipient penetration (Figs 1A, 2A, 3A, arrowheads). Abundant lomasomes were also seen in symbiont cells at zones of contact where penetration pegs were not evident in those planes of section (Figs 2 & 3, arrowheads). In Fig. 1E, a concentrically folded membranous structure was positioned in the fungal cytoplasm directly behind the wall protrusion. In Fig. 3A & B, a larger, clearly membrane-bounded vesicle or plasmalemma fold appeared just outside the fungal protoplast immediately adjacent to the wall protrusion. A similar structure, separated within the plane of section from the protoplast by a thin bridge of wall material, was also seen in an algal cell adjacent to fungal cell contact where the algal wall appeared thickened (Fig. 3E & F). Such structures were observed repeatedly. The boundary between contacting fungal and algal cell walls often had a sinuous, corrugated appearance (Fig. 3C & D). Not infrequently, wall material from both symbionts appeared to intermix irregularly, making the exact boundary between fungal and algal cell walls unclear (Fig. 3A).

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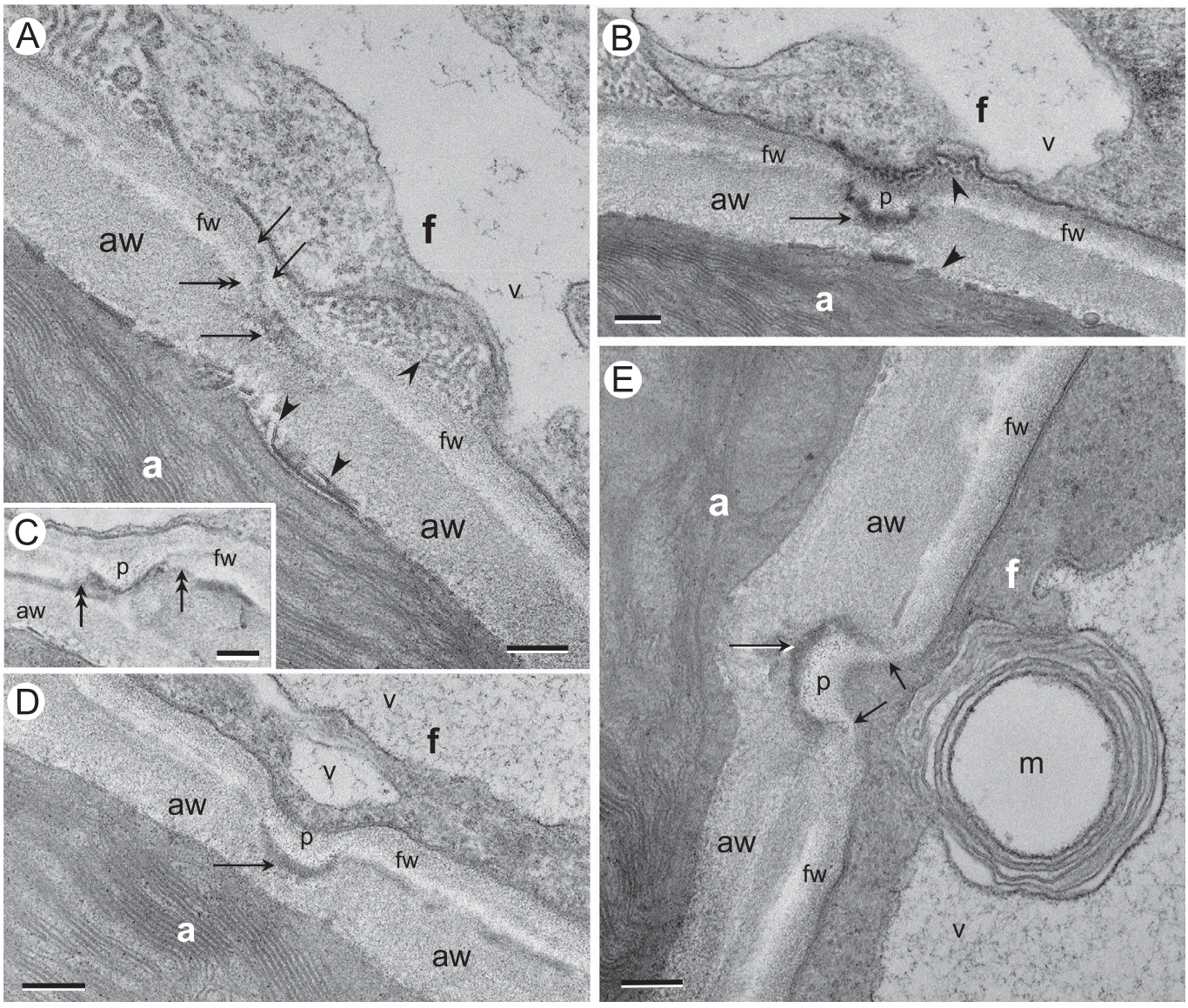

Figure 1. TEM images of symbiont contact zones between *Endocarpon pusillum* (f) and its phycobiont *Diplosphaera* (a). Developmental stages of intraparietal haustorium formation. A, initiation of haustorium from new inner layer of fungal cell wall (oblique arrows) protruding through break in outer wall layers (double arrows). Single horizontal arrow indicates zone of algal cell wall dissolutions. Note lomasome-like membranous inclusions (arrowheads), vesicular in fungal cell and tubular in algal cell, exterior to respective plasmalemmas and continuous with cell wall materials. B, protrusion into area of algal wall disruption (single horizontal arrow); arrowheads indicate lomasome-like membranous inclusions. C, thickening of incipient penetration peg emerging from rupture in outer fungal cell wall layers (double arrows). D & E, further intrusion and thickening of incipient haustorium into area of algal cell wall dissolution (horizontal arrow). Note continuity of peg base with innermost layers of fungal cell wall (oblique arrows in E). In E, plasmalemmasome-like folds of concentric membranes (m) are positioned just behind the haustorium within the fungal cell. Abbreviations: a = algal cell; f = fungal cell; aw = algal cell wall; fw = fungal cell wall; m = plasmalemmasome-like concentric membranous folds; p = fungal wall protrusion/peg-like haustorium; v = vacuole. Scales: A–E = 200 nm.

Microfruticose/isidiose crustose Porina microcoralloides *and its* Trentepohlia *phycobiont*

Symbiont contact zones often consisted of a broad protuberance of the fungal cell that made a concave deformity in the surface of the algal symbiont cell (Fig. 4A, C & D). Reduction of the algal cell wall was moderate (Fig. 4A & D) to substantial (Fig. 4C). The fungal cell wall, elsewhere several hundred nm in thickness, was reduced to less than *c.* 50 nm at the contact zone (Fig. 4C & D), and in some instances could scarcely be distinguished (Fig. 4B). The fungal wall protrusion arose from inner wall layers that emerge through a break in the outer wall layers (Fig. 4C, double arrow). At some interfaces, the fungal cell surface was itself concave where the contacted algal symbiont protruded into the fungal cell (Fig. 4B), exactly reversing the expected positional roles of the two symbionts. As with the fungal haustorium, the algal protrusion was continuous with and clearly arose from the innermost wall layer(s) that appeared to have been laid down secondarily (Fig. 4B) and had broken through the outer layers.

Microfruticose/isidiose Porina nanoarbuscula *and its* Trentepohlia *phycobiont*

At symbiont contact zones, the fungal cell often showed a haustorium-like protuberance of variable size that produced a concave deformity in the cell surface of the contacted alga (Fig. 5A–C). However, in some cases the fungal contact surface was concave (Fig. 5D), with the cell wall highly reduced in thickness at the contact zone, often to

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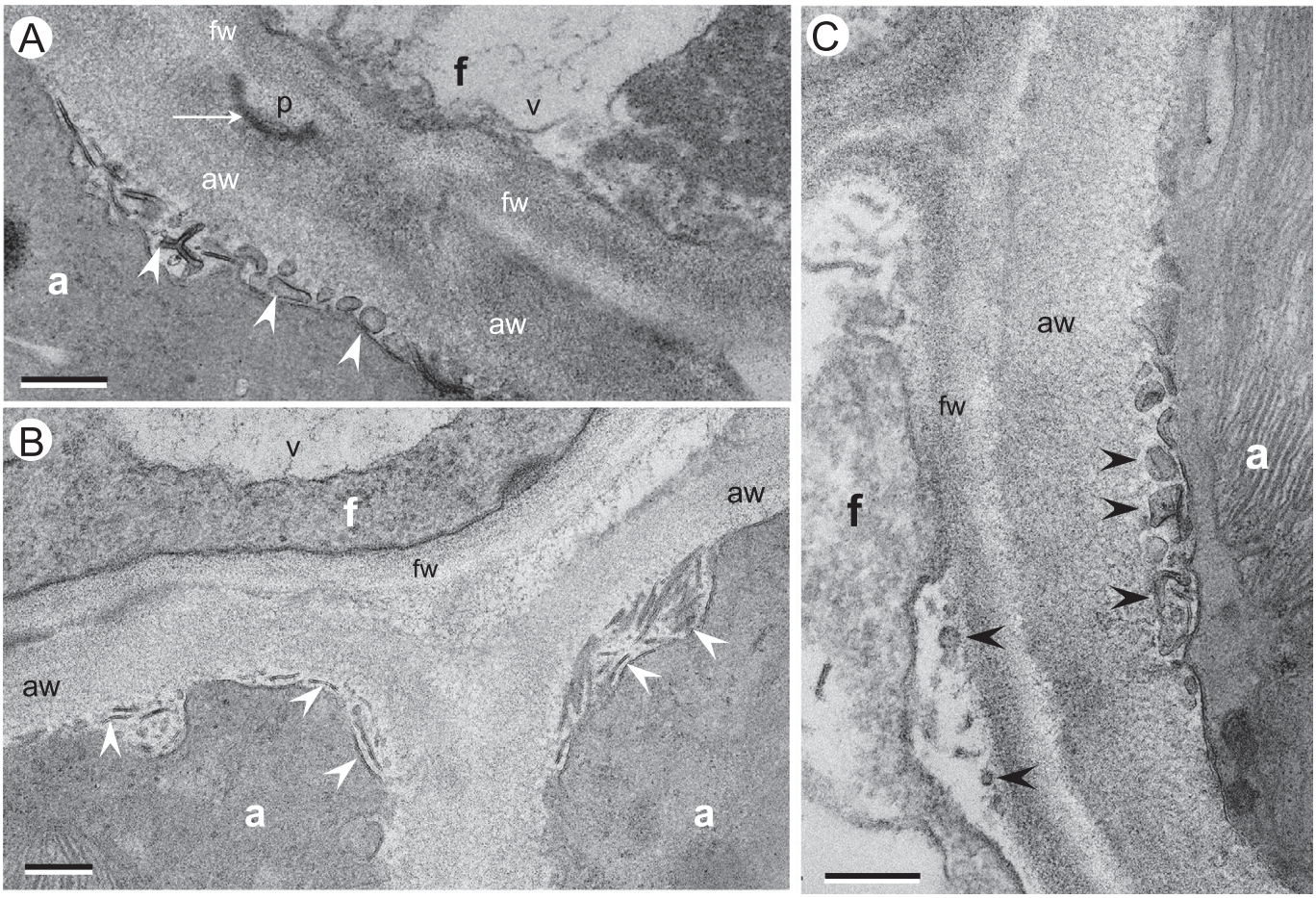

Figure 2. TEM images of symbiont contact zones between *Endocarpon pusillum* (f) and its phycobiont *Diplosphaera* (a). Abundant vesicular and tubular inclusions (arrowheads) between algal plasmalemma and cell wall, also in fungal cell in C; note thickening of algal wall. Horizontal arrow in A indicates area of algal cell wall dissolution. Abbreviations: a = algal cell; f = fungal cell; aw = algal cell wall; fw = fungal cell wall; p = peg-like intraparietal haustorium. Scales: A–C = 200 nm.

the point of indistinguishability. Algal cell walls were also often reduced at the contact zone (Fig. 5B & C).

Crustose foliicolous Byssoloma kakouettae *and its trebouxiophycean phycobiont*

The fungal cell as a whole pushed broadly into the algal cell, which it often deformed into a crescent-shaped outline in section (Fig. 6A & C). There was no distinctive structure that could be recognized as a haustorium. At the contact zone, the fungal cell wall was reduced to no more than 50–100 nm in thickness (Fig. 6B–D). The contacted algal cell wall also showed a reduction in thickness.

Other crustose foliicolous lichens

Byssoloma leucoblepharum cells bulged into a broad concavity made in the contacted cells of its trebouxiophycean phycobiont. The fungal cell wall was sometimes reduced to the point of indistinguishability in the contact zone; deposition of algal wall materials in apposition to the protrusion was evident (Fig. 7A). *Calopadia puiggarii* cells protruded broadly against those of it phycobiont *Heveochlorella* (Fig. 7B); its cell wall was reduced to less than 100 nm in the contact zone. *Fellhanera bouteillei*, in chiefly appositional contact with its phycobiont *Chloroidium*, showed a reduction of its cell wall to a thickness of *c.* 50 nm (Fig. 7C & D).

Discussion

Haustorial penetration into the algal cell lumen was not seen in any of the microlichens examined in the present study, including the foliicolous taxa that have little or no differentiation of thallus tissue layers (Sanders & de los Ríos 2016, 2023). All exhibited intraparietal penetrative contacts between symbionts, although of quite varied morphologies, ranging from narrow, peg-like wall protrusions to broad, undifferentiated portions of the fungal cell surface. The former are comparable to the intraparietal haustoria described in other lichen-forming taxa, while the latter invaginate or deform the phycobiont cell directly without elaboration of any distinctive penetrative structure. Degradation of the algal cell wall at the symbiont interface, with concomitant thinning of the fungal cell wall, qualifies these broad contacts as intraparietal even though no differentiated haustorium can be recognized. Studies of plant pathogenic fungi have likewise reported a wide variety of invasive contacts, for which the definition of haustorium was found to be insufficient to encompass the range of structures to which this term has been applied (Calonge 1969).

The haustoria we observed in *Endocarpon pusillum* resemble one depicted in a previous ultrastructural survey of that taxon (Ahmadjian & Jacobs 1970). They are minute, intraparietal pegs formed from a newer, interior fungal wall layer(s) that breaks through the outer layers, as observed in other lichen-forming taxa

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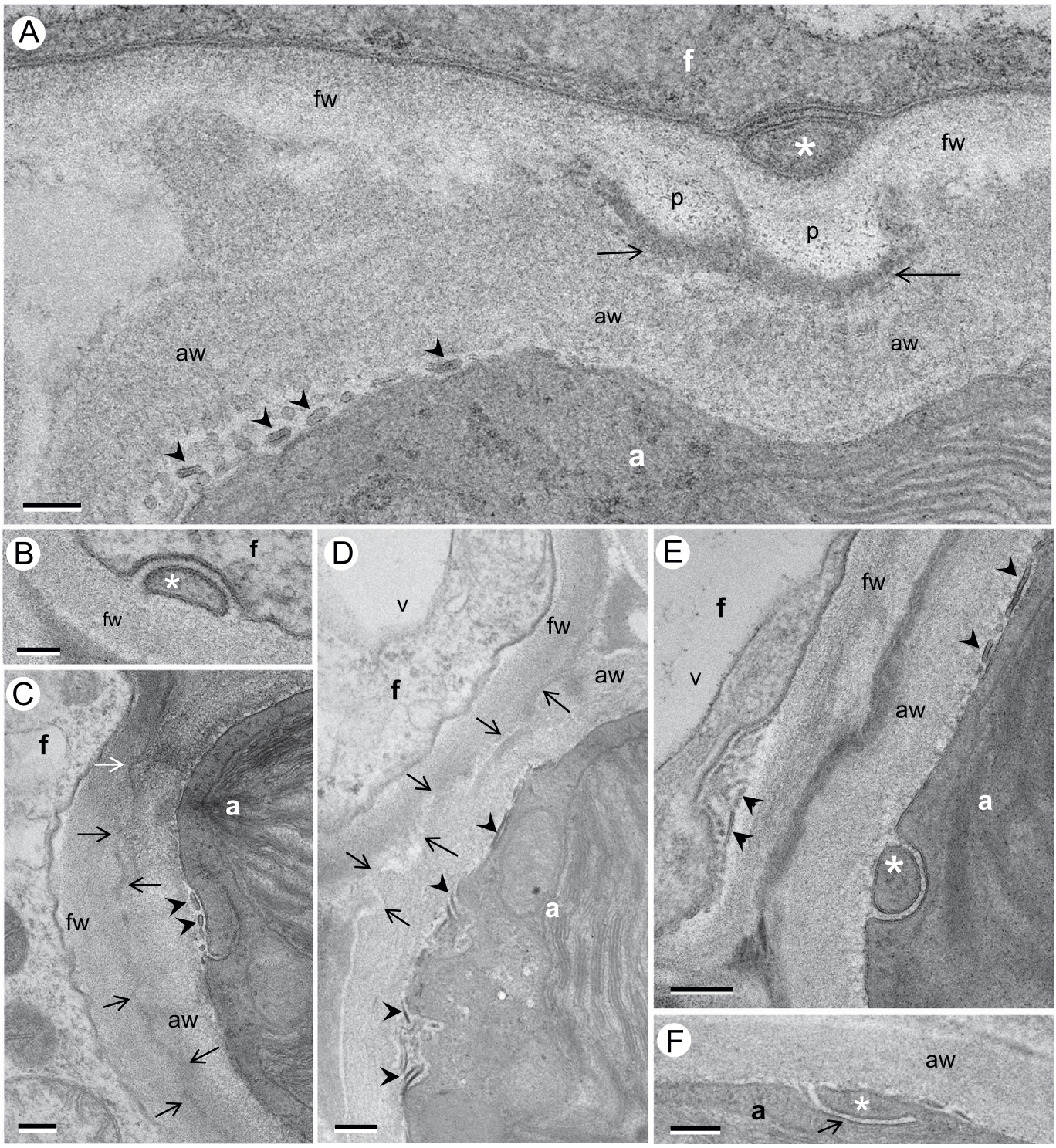

Figure 3. TEM images of symbiont contact zones between *Endocarpon pusillum* (f) and its phycobiont *Diplosphaera* (a). A, broad boundary showing two fungal pegs to the right and highly thickened, indistinctly intermixed fungal and algal wall materials to the left. Horizontal arrows indicate region of algal cell wall degradation ahead of fungal penetration pegs, directly behind which an exterior elaboration of the fungal plasmalemma is evident (asterisk). Arrowheads indicate smaller, lomasome-like vesicles/tubules emerging from algal plasmalemma and embedded within wall material. B, asterisk indicates elaboration of the fungal plasmalemma similar to the previous micrograph; the material separating it from the rest of the protoplast is clearly continuous with and part of the fungal cell wall. C & D, sinuous contact zones (arrows) between fungal and algal walls. Arrowheads indicate lomasome-like vesicles and tubules between protoplast and cell wall of algal symbiont in contact region. E, asterisk indicates exterior elaboration of algal protoplast, similar to that shown for the fungus in A and B, with a thin layer of wall material clearly separating it from rest of protoplast, at least within this plane of the section. Arrowheads indicate lomasome-like vesicles and tubules between the protoplast and cell wall of both symbionts in the contact area. F, formation of bridging wall material (arrow) similar to that shown in previous micrograph, in longitudinal view or in process of development. Abbreviations: a = algal cell; f = fungal cell; aw = algal cell wall; fw = fungal cell wall; p = peg-like intraparietal haustorium. Scales: A & B = 100 nm; C–F = 200 nm.

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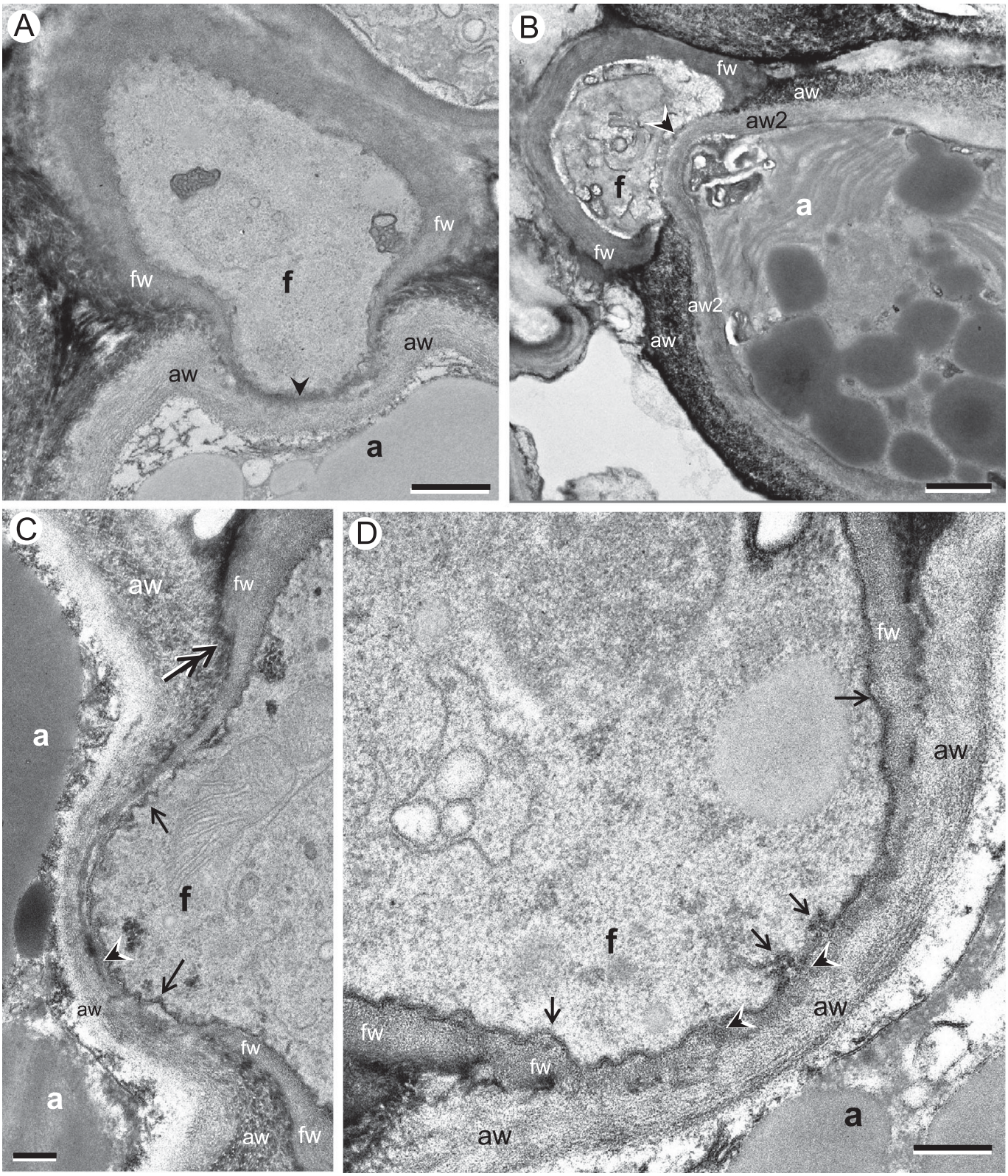

Figure 4. TEM images of contact zones between lichen-forming fungus *Porina microcoralloides* (f) and its algal symbiont *Trentepohlia* (a). In A, C and D, a broad, thin-walled bulge in the fungal cell produces a concave deformation in the algal cell surface, with thinning of the algal cell wall (especially in C and D), while in B the algal symbiont protrudes into the fungal cell, whose wall is reduced to a faint remnant within the zone of contact. Note algal cell protrusion arising from inner wall layer (aw2). Arrowheads indicate remnant of fungal cell wall surrounding algal protrusion; arrows in C & D indicate fungal plasmalemma. Double arrows in C indicate rupture in outer fungal cell wall as inner layer emerges as penetrative bulge. Abbreviations: a = algal cell; f = fungal cell; aw = algal cell wall; fw = fungal cell wall. Scales: A = 500 nm; B = 1 μm; C & D = 250 nm.

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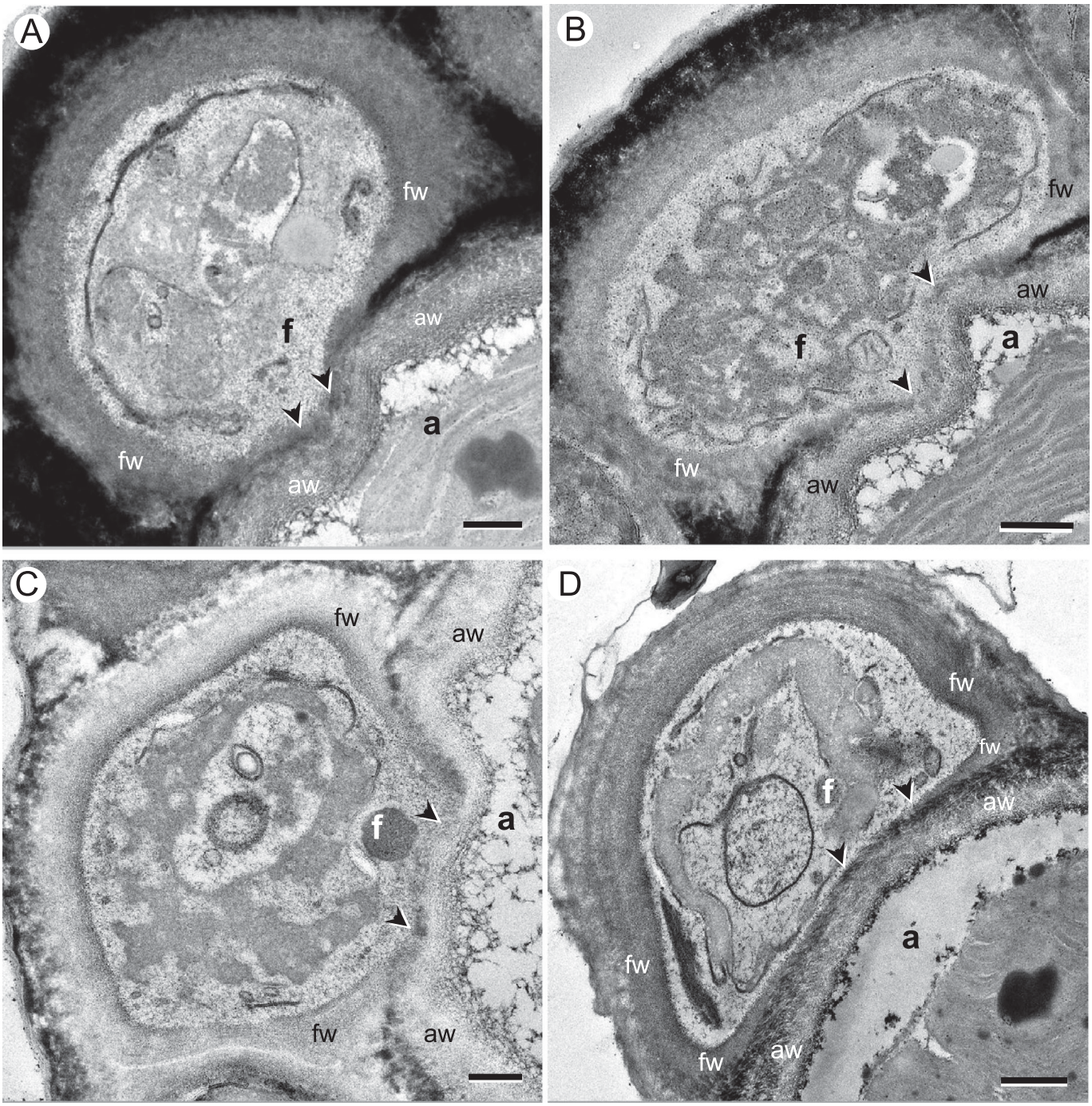

Figure 5. TEMs of contact zones between lichen-forming fungus *Porina nanoarbuscula* (f) and its algal symbiont *Trentepohlia* (a). Arrowheads indicate remnant fungal cell wall. Abbreviations: aw = algal cell wall; fw = fungal cell wall. Scales: A & C = 250 nm; B & D = 500 nm.

such as the basidiolichen genus *Dictyonema* (Oberwinkler 1980). In our observations, these haustoria are always enclosed within a fungal cell wall. The inner wall layer from which the haustorium arises is relatively thin initially, but soon thickens, particularly at the haustorial tip (Figs 1C–E, 3A). Vacuoles are usually present in the vicinity, and one referee of this manuscript suggested the possibility that they might be involved in generating the turgor pressure for wall protrusion. We find the intraparietal haustorium of *E. pusillum* to be quite similar morphologically to those observed in other *Verrucariaceae*, such as *Dermatocarpon miniatum* (L.) W. Mann (Galun *et al.* 1973; figs 1–7) and *Verrucaria* spp. (Kushnir & Galun 1977; figs 1–5), although we disagree with those authors' interpretation of the haustorium as a naked protoplast. The frequent presence of plasmalemmasomes, lomasomes or lomasome-like vesicles and tubules localized at contact zones in cells of both symbionts is suggestive of active construction and/or modification of cell walls. Lomasomes were first noted in early TEM studies of fungi as plasmalemma-derived elaborations exterior to the cell and associated with cell wall materials (Girbardt 1958). They were also observed in similar contexts within green algal cells (Barton 1965; Crawley 1965), and in the lichen-forming fungus *Peltigera canina* (Boissière 1982; Lallemant *et al.* 1986). The term plasmalemmasome has also been

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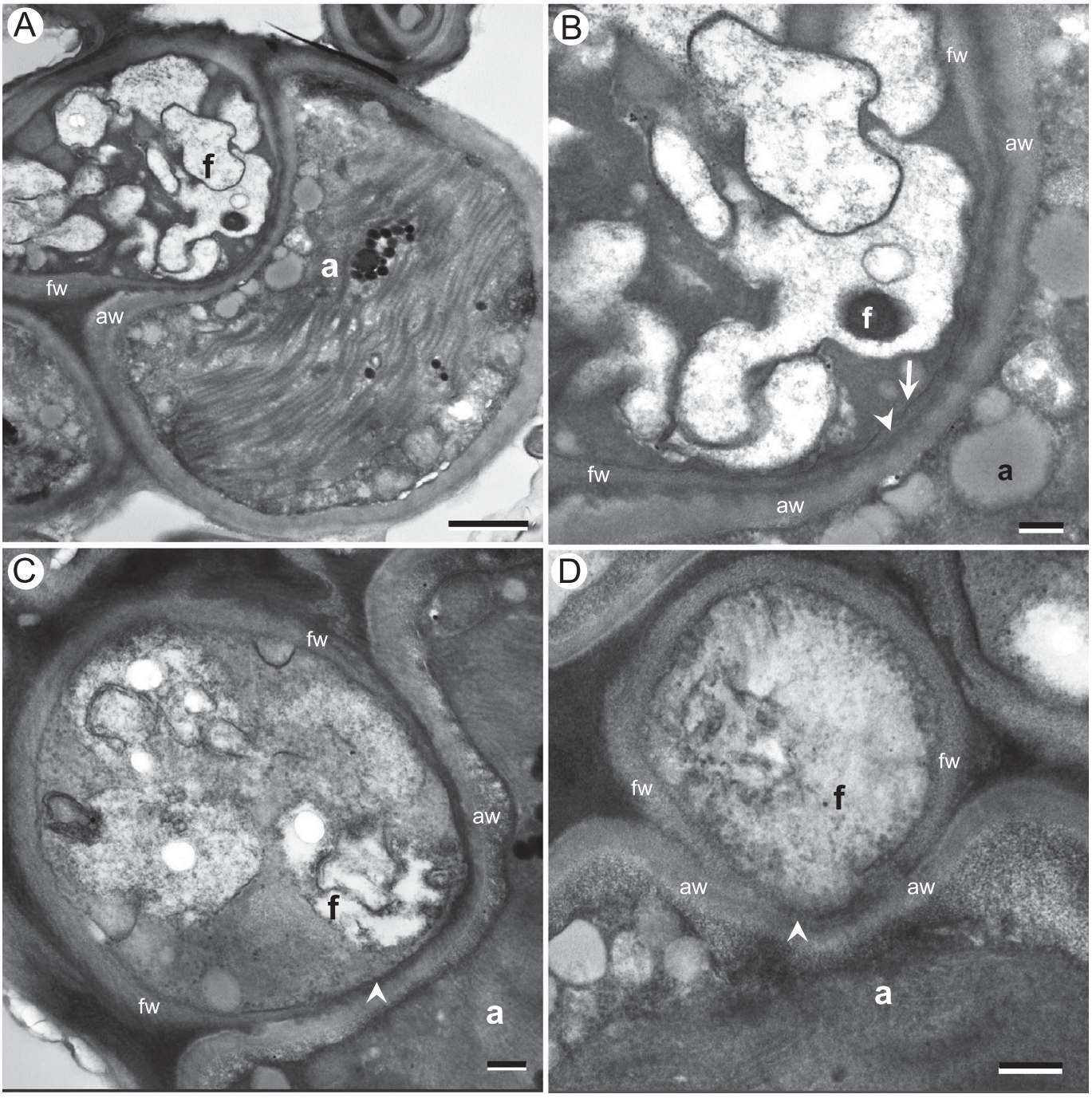

Figure 6. TEM images of symbiont contacts between the lichen-forming fungus *Byssoloma kakouettae* (f) and its trebouxiophycean algal symbiont (a). B shows the contact zone of A at higher magnification. Arrowheads indicate remnant fungal cell wall; arrow in B indicates fungal cell plasmalemma. Abbreviations: aw = algal cell wall; fw = fungal cell wall. Scales: A = 1 μm; B–D = 200 nm.

applied to folded modifications of the cell membrane. According to Marchant & Moore (1973), plasmalemmasomes differ from lomasomes in that the former are not surrounded by wall materials. Although certain membrane elaborations observed in TEM have been shown to be artifacts of chemical fixation and processing (e.g. Büdel & Rhiel 1987), lomasomes and plasmalemmasomes are evident in cryofixed as well as chemically processed samples, and therefore appear to be genuine features of living cells (Marchant & Moore 1973). Their precise functions have not been clarified but seem to be associated with wall growth and modification (Wilsenach & Kessel 1965). They may also play a role in the recycling or removal of excess membrane involved in such growth processes (Mazheika *et al.* 2022), which may add more membrane to the plasmalemma through exocytosis than can be accommodated by the rate of cell enlargement (Riquelme *et al.* 2018). In *Endocarpon*, the visible integration of lomasome-like membranous structures among the innermost layers of wall material in contacting cells of both symbionts (Figs 1A & B, 2, 3A), and the larger, single elaborations of the

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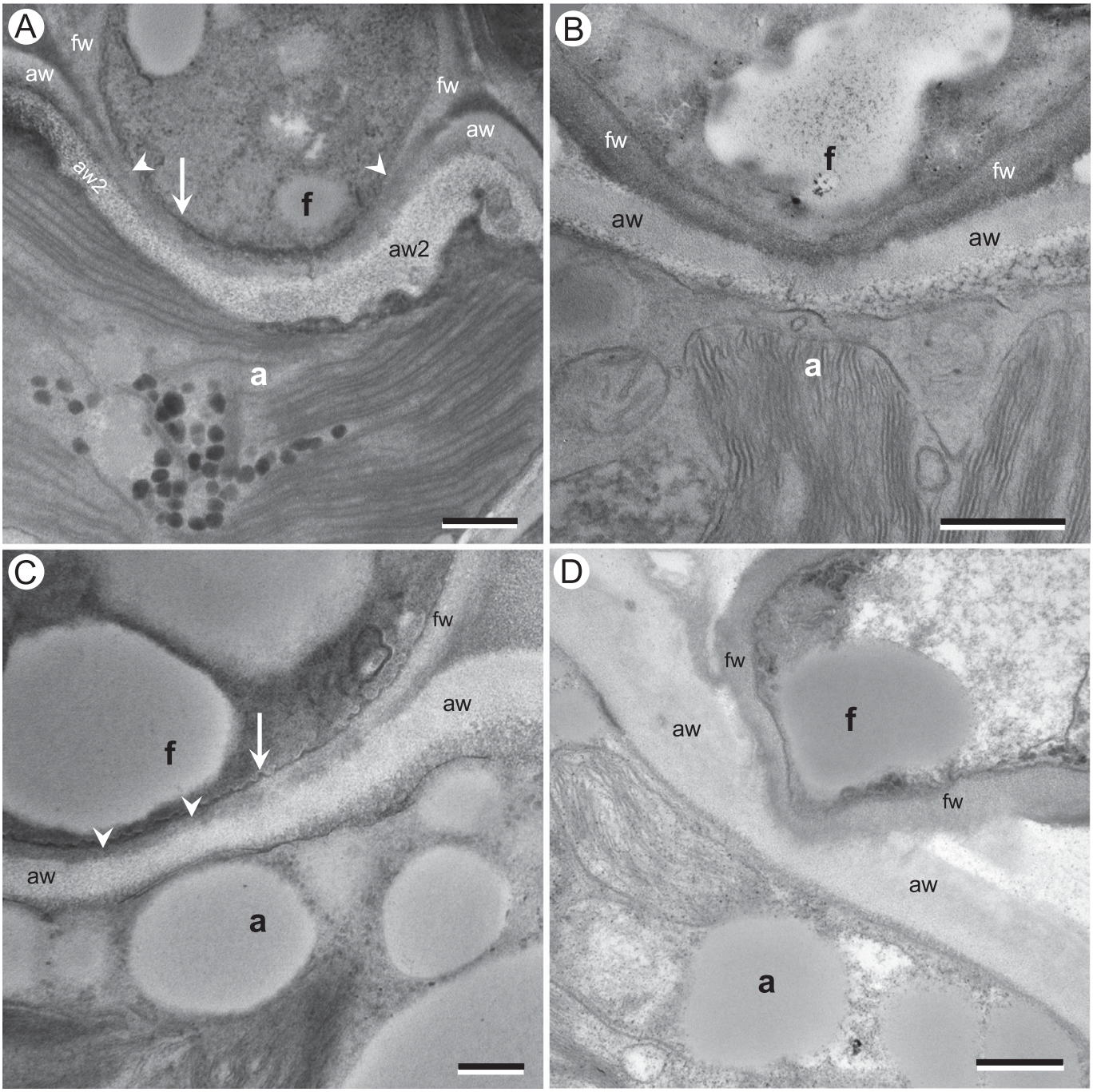

Figure 7. TEM images of symbiont contacts between three foliicolous lichen-forming fungi and their 'Watanabealean' (*Trebouxiophyceae*) algal symbionts. A, *Byssoloma leucoblepharum.* B, *Calopadia puiggarii.* C & D, *Fellhanera bouteillei.* Arrowheads indicate remnant fungal cell wall; arrows indicate fungal cell plasmalemma. Abbreviations: aw = algal cell wall; fw = fungal cell wall. Scales: A, B & D = 500 nm; C = 200 nm.

plasmalemma bridged by material clearly continuous with the cell wall (Fig. 3A, B, E & F) suggest a role in cell wall construction. Vesicle remnants within the mycobiont cell wall have also been noted (Büdel & Rhiel 1987) and may represent a subsequent stage of this process. The membranous elaborations are particularly evident where cell wall thickening is visibly taking place. Deposition of encapsulating algal wall material in apposition to fungal penetrative structures has been observed at contact interfaces in many other lichen taxa (Geitler 1934; Tschermak 1941; Honegger 1984), as well as plant hosts of fungal pathogens (Bracker & Littlefield 1973). The contacting cells at the symbiont interface in *Endocarpon* thus appear to be at least as much involved in wall deposition as they are in its degradation. The thickening of the short, narrow, intraparietal peg, as well as the algal cell wall in apposition to it, are not suggestive of adaptations likely to accelerate solute transfer across the contact interface. From a phylogenetic perspective, such interfaces might be interpreted as arising from a dynamic equilibrium between fungal invasion and algal defence that stabilizes the lichen symbiosis, as previous authors have proposed. Haustorial penetration of algal prey, a pre-symbiotic means of obtaining resources from a victimized cell, may have lost

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its original function when the need for extractive organs was obviated by the evolution of algal carbohydrate release. A separate but equally interesting question is whether those peculiar peg-like intrusions also have some functional significance in their present form.

We propose that such structures may serve an important function in firmly anchoring the symbiont cells to each other at their contact zones. As poikilohydric partnerships, lichens typically undergo daily cycles of hydration and desiccation, with concomitant extremes of swelling and shrinkage in tissues and cells. These forces can easily rupture tissues, which in some cases contributes significantly to lichen morphogenesis. Examples include the tissue perforation in the lace lichen (Lutz 1894; Peirce 1898; Sanders 1989) and fissuring of the epinecral layer in *Lasallia* (Sanders & de los Ríos 2018), as well as the characteristic cracking of the cortex in many saxicolous crusts (Brodo *et al.* 2001; Sanders *et al.* 2004), particularly evident in the splitting of thallus units into secondary areolae (Létrouit-Galinou & Asta 1994). At the cellular level, such stresses risk pulling fungal and algal cells apart at their junctions, potentially rupturing the layer of hydrophobic sealant that canalizes solute transfer between them. In the intertidal lichen *Wahlenbergiella tavaresiae* (R.L. Moe) Gueidan *et al.* (also a member of the *Verrucariaceae*), the intraparietal haustorium bears lateral flanges that are embedded within the wall of the phycobiont like the barbs of a spear, ensuring that symbiont cells are held together in close contact (Sanders *et al.* 2004). In *Endocarpon*, contacting walls of fungal and algal symbionts are often interlocked in sinuous, corrugated borders (Fig. 3C & D), also evident in a published micrograph of endolithic *Verrucaria* sp. (Kushnir & Galun 1977; fig. 5). In other places, mutual construction or reconstruction intermeshes fungal and algal cell walls such that the exact boundary between them is not readily distinguishable (Fig. 3A). These features suggest further adaptations to integrate adjacent symbiont cells in resistance to separating stresses. Malachowski *et al.* (1980) viewed the peg-like intraparietal haustoria they observed in *Usnea cavernosa* as serving to 'hold algal cells close as thallus matures and air spaces develop'. That haustoria can effectively maintain physical unification of symbiont cells is corroborated by the observations of Honegger (1985). She reported that *Coccomyxa* algae, which are completely surrounded but not penetrated by hyphae of their *Peltigera* and *Solorina* partners, can be easily separated from the mycobiont in the laboratory, whereas no pure fraction of phycobionts can be obtained from lecanoralean mycobionts that penetrate their trebouxioid algal symbionts with intracellular or intraparietal haustoria. In lichens with autospore-forming *Trebouxia* as phycobiont, fungal penetration and separation of dividing algal cells is thought to play a mechanical role in distributing algal symbionts in coordination with mycobiont growth (Greenhalgh & Anglesea 1979; Honegger 1987).

Benefiting in part from the improved resolution in electron microscopy over recent decades, our interpretation of the intraparietal haustorium found in *Endocarpon* and *Verrucariaceae* differs from that offered in previous works (Galun *et al.* 1971*b*, 1973; Kushnir & Galun 1977; Galun 1988). On the other hand, the extreme level of wall thinning we observed in other taxa suggests that a naked haustorial protoplast in lichen-forming fungi may not be a far-fetched idea. In the microfruticose/isidiose *Porina* spp. and the foliicolous taxa examined (Figs 4–7), the cell wall of the fungal protrusion was often substantially reduced to no more than 50 to 100 μm in thickness, and in many instances was hardly distinguishable (Figs 4B & C, 5B–D, 6C & D, 7A). However, the protrusion was always in direct contact with the algal cell wall, with no space visible between them. The actual thickness of the fungal cell wall is likely to be even less than that estimated from micrographs, since only those sections that are exactly perpendicular to the wall will display its true thickness, while oblique sections will exaggerate it. Somewhat peg-like protrusions were occasionally observed in these lichens (Fig. 7D), but in general their symbiont interfaces tended to involve broader surfaces without differentiated haustoria. These more extensive contact surfaces, in combination with the highly reduced cell walls, appear more likely to facilitate solute exchange than the peg-like haustoria, and would seem somewhat less suited to maintaining physical unity of symbiont cells. Before interpreting the highly thinned walls as any sort of adaptation, however, one should keep in mind that the protrusion is initiated from an inner wall layer that, at least initially, will be only a fraction of the wall's total thickness.

Between lichen symbionts, protrusion and penetration can be reciprocal (Figs 4B & 5D). Geitler (1933) noted a counter-intrusion by cyanobiont cells into the appressoria of *Lempholemma chalazanum* (Ach.) B. de Lesd., which he interpreted as an indication that the alga fights back against fungal attack. Finger-like projections observed in cyanobiont cells squeezed free of mycobiont hyphae in *Thyrea pulvinata* (Schaer.) A. Massal. (Geitler 1936; fig. 4h) probably also correspond to such reciprocal intrusions of the algal symbiont into the fungal partner. In a published TEM micrograph, the *Gloeocapsa*-like symbiont of *Gonohymenia mesopotamica* J. Steiner can be seen penetrating a cell of the mycobiont (Paran *et al.* 1971; fig. 1, lower right), although it is not commented upon in the text. Penetrative reciprocity was noted in contact zones between the intertidal lichen-forming fungus *Wahlenbergiella tavaresiae* and its phaeophycean phycobiont *Petroderma*, which showed interdigitating haustoria-like interpenetrations, with concomitant thinning of both algal and fungal cell walls (Sanders *et al.* 2004; figs 40–42). As with their mycobionts, the algal symbionts *Petroderma* and *Trentepohlia* are filamentous, that is, with polar growth in a linear trajectory that can be directed intrusively. Recent genomic studies in trebouxiophycean phycobionts describe genes coding for enzymes that digest fungal wall polymers such as lichenan, which the authors speculate might be used by the alga as an additional source of glucose (Puginier *et al.* 2024). Such results at least suggest an enzymatic basis for reciprocal penetration. If it seems surprising that the alga should be stimulated to grow into fungal cells, we should recall that maintaining a functional contact interface is also of importance to the algal partner. Yet the current system of classifying symbiont contacts in lichens (appositional, intraparietal, intracellular) only takes into account the degree to which the algal cell wall is breached, while ignoring any dissolution of that of the mycobiont. To a large extent this reflects the expectation that the mycobiont will play an offensive role and the algal symbiont a defensive one at the cellular level. This longstanding idea may require some reconsideration.

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