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ON THE SPORES (INCLUDING GERMINATING SPORES) OF *HORNEOPHYTON (HORNEA) LIGNIERI* (KIDSTON & LANG) BARGHOORN & DARRAH (1938). [a]

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Abstract

Spores isolated from the sporangium of *Horneophyton lignieri* have been examined and their morphology have been described and compared with dispersed spore types. A single sporangium of the plant contain two types of spores. Information has been obtained regarding their size ranges and variations under different conditions of preparation.

Germinating spores originally reported by Lyon (1957) have been re-investigated and few of them have been identified as belonging to *Horneophyton lignieri*. Probable early developmental stages of gametophytes formed by these spores have been recognized.

Introduction

Kidston & Lang (1920) in their account of vascular plants of the Rhynie chert recorded the general appearance and average size of spores of *Rhynia gwynnevaughani* and *Horneophyton (Hornea) lignieri* which they found in ground sections of sporangia. The spore size of *Horneophyton lignieri* is ca 50μ.

Lyon (1957) described a few isolated spores in the matrix which appeared to to have germinated just before petrifaction. The outgrowths from these apparently represented early stages in the development of gametophytic generation of some of the Rhynie plants. Largely on the basis of estimated unsplit size, Lyon suggested that some of the spores (67μ in diameter) might belong to *Rhynia major* in appearance and a few spores which were upto 81μ and resembled those of *Rhynia major* in appearance, might also belong to the same plant. In making his observation, however, he did not take into account of possible dimensional changes due to swelling after shedding and in any case information regarding size ranges was not at that time available to him.

Studies on Devonian spores have been covered on dispersed compressed specimens isolated from cores or other samples of sedimentary rocks. In only a few cases, spores have been isolated from the sporangia of known plants and assigned to form genera. A list of which has been given in Banks (1968). Although several workers, Kidston & Lang (1920), Lyon (1957) and El-Saadwy (1966, unpublished thesis) have made observations on the spores of Rhynie plants, no attempt has been made to isolate and "type" them and study their variability.

Suitable material being available, it was decided to attempt to:

(a) isolate spores from individual sporangia, critically examine and describe the spores of *Horneophyton lignieri* and compare them with established spore types.

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(b) obtain information regarding size ranges and other variable features of both shed and unshed spores under different conditions of observation,
(c) examine critically and identify where possible the germinating spores in slides prepared by Lyon (1957).

Materials and Methods

The materials used in the present study were collected by Dr. Lyon who handed them over to me for further investigations. The material consisted of the following:

(a) A selection of blocks containing sporangia of *Horneophyton*.
(b) Seventeen chip preparations Ref: (RLS.I-17) containing germinated spores together with the broken block in which they were found.

The following methods were employed:

(a) *Chemical and Mechanical:*

(i) *Location and selection of sporangia*

Sporangia were located by examining wet block surfaces under a stereo binocular microscope. Identification presented little difficulty after some experience had been gained and in the case of *Horneophyton lignieri* sporangia, microscope examination was only necessary to confirm identification by naked eye. Sporangia which contained abundant spores and were otherwise well preserved, were normally selected and their position heavily marked with a white wax pencil. The block containing the selected sporangium was then shattered on a firm, clean surface and fragments of reasonable size, containing parts of fructification were subsequently identified by the marking they bore. The search for these was continued until at least two fragments of reasonable size were recovered. One (after trimming off the surplus chert) was used for spore isolation, the other for making peel and ground sections for comparitive purposes.

(ii) *Material for germinating spores*

As previously stated, 17 prepared slides were provided by Dr. Lyon and a few additional specimens were obtained by examining fragments from the remains of the original block under the microscope in xylol.

(iii) *Isolation of Spores*

The spore mass in a fragment of sporangium or material containing dispersed spores was boiled in water for five minutes to remove any external contamination and soluble mineral matter. The water was then decanted and the material transferred into a polythene tube, enough 20% Hydrofluoric acid (usually 5-10 c.c.) being added to the fragment. About 1 c.c. concentrated Hydrochloric acid was also added to check gel formation, the tubes were covered with lids and left in a fume chamber for a period of one week. At the end of this period, the acidic layer was removed very gently by decantation. Hydrofluoric acid treatment was then repeated for another week. Subsequently, the Hydrofluoric acid was decanted and the residue centrifuged and washed several times with water before final treatment with Schultze's solution for 24 hours.

(iv) *Chlorination*

Small portion of the residue (in water) containing spores was transferred to centrifuge tubes and chlorinated using a mixture of concentrated Sulphuric acid (5-10 c.c.), Glacial acetic acid and saturated Potassium chlorate solution (2 c.c. each). In preparing this mixture, it was found that the Acetic acid was best added first to the saturated Potassium chlorate solution and thereafter, the Sulphuric acid dropwise, until a bright yellow colour developed to its maximum intensity. Before this the reagent was allowed to stand for about one minute. Experience showed that the process of chlorination must not exceed 4-5 minutes, otherwise damage occurred to the spores. At the end of this period, the acidic layer was decanted and the residue washed several times in water. Well preserved spores tolerant of dehyderation were mounted in hot Canada balsam as described by Schopf (1960) with the exception that the spores were mounted on standard microscopic slides instead of using double cover glass "Sandwiches" Fragments containing germinating spores were mounted, preferably in 'Duramount' 244, between two cover slips on reversible "Duralumin" microscope slides of the type described by Sims & Lyon (1963).

(b) *Ground & peel sections of sporangium*

Ground and peel sections of sporangium were prepared as mentioned earlier (Bhutta, 1972).

Results and Discussion

(a) Morphology of sporangium of *Horneophyton lignieri*

Sporangia of *Horneophyton* are widely distributed and relatively abundant in the Rhynie Chert. Sporangia are cylindrical columellate, terminal on ultimate branches, rather variable in size (generally about 2 mm. long and 1-2 mm. broad), apical region somewhat flattened (cf. Kidston & Lang, 1920) in most of the specimens or concave in dehisced sporangia (cf. E -Saadwy, 1966) or convex (cf. Bhutta, 1972). Wall of the sporangium is about 0.25 mm. in thickness. The outer tangential and radial epidermal cell walls are somewhat thickened and bounded by a cuticle. Underlying the epidermis is a "middle layer" of thin walled parenchyma (usually poorly preserved) but very often about six cells in width to the inside of which lies a narrow persistent "tapetal" region forming the boundry of the spore sac and extending over the central columella (sometimes branched) composed of elongated thin walled cells (also cf. Kidston & Lang 1920; plate IX, figures 59,60,62 & 64).

Description of spores:—(Based on isolated specimens)

Spores brown both in ground and peel sections and slightly darker after isolation (unbleached), very dark when found dispersed in the matrix. Triradiate, spherical, uncompressed Laesurae distinct, slender to sinuous, dark coloured, slightly thickend and raised (figs. 1-2 and text-fig. 1), commissure extending almost to the periphery. Lips indistinct Proximal face a three sided pyramid, smooth to thinly ornamented with cones. Some of the spores in lower size range show radially arranged cones on proximal surface (figs. 3-4 & text-fig. 2). Distal surface sually ornamented with erect pointed cones $\pm$ I μ thick at their bases. Cones variable and less numerous, indistinct particularly in most of the isolated specimens, although often persistant in ground sections (damage occurring during isolation). Curvaturae perfectae arc shaped

Explanation of text figures 1-10.

Apiculiretusispora plicatus **ALLEN**

Fig. 1. Oblique proximal view showing curvaturae and distal coni at equatorial line. X 400

Fig. 2. Shows raised laesurae and distinct contact area. Curvaturae perfectae is well marked. X 375.

Fig. 3 & 4. *Emphanisporites decoratus* Allen showing radially arranged thickenings at proximal surface in fig. 3 while radially arranged coni on proximal surface in fig. 4. X 400.

Germinating spores

Fig. 5. Preliminary stage in germination, no cellular outgrowth having as yet developed (cf. text-fig. 3) X 350.

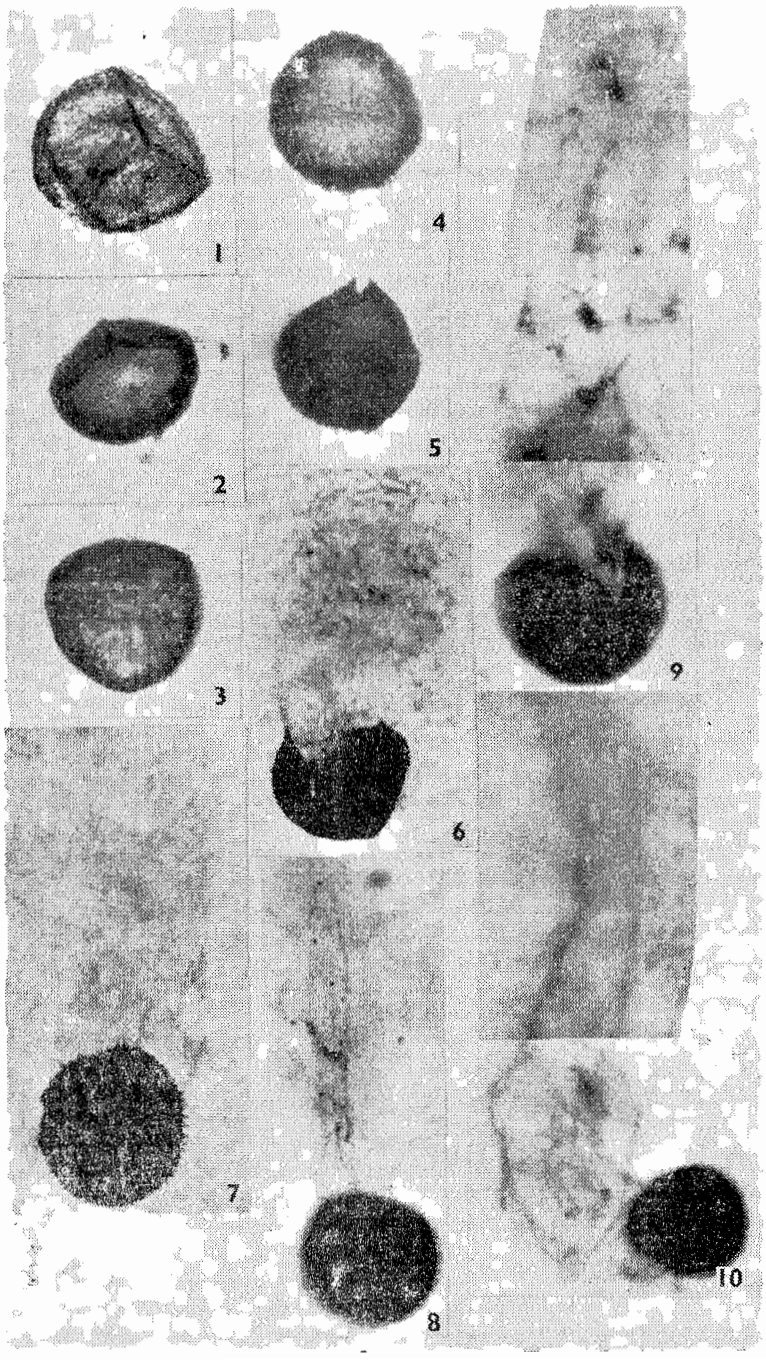
Fig. 6. An obovate multicellular outgrowth composed of rather squarish cells has developed. (cf. text-fig. 4) X 250.

Fig. 7. Cellular details are imperfectly preserved in the outgrowth. (cf. text-fig. 5) X 350.

Fig. 8. Probably the outgrowth has undergone shrinkage and fungal attack (cf. text-fig. 6). X 300.

Fig. 9. A well developed but with fewer "coronal" cells (cf. text-fig. 7) than the specimen in fig. 10. X 250.

Fig. 10. The largest outgrowth seen so far with well developed "Coronal" cells (cf. text-fig. 8). X 200.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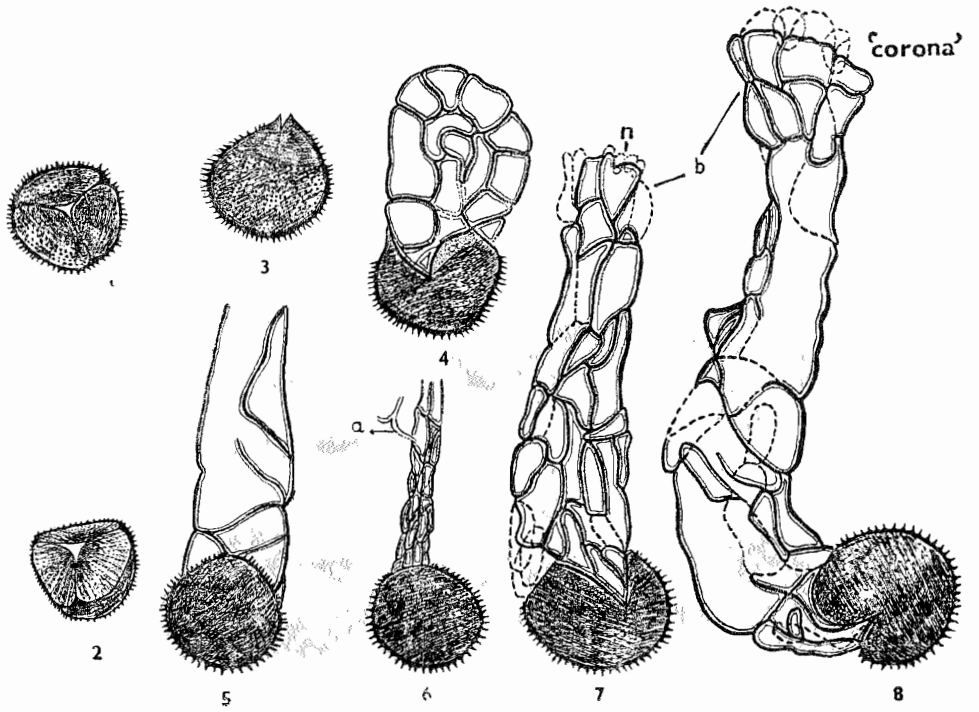


Text-fig. 1.—Camera lucida drawing of *Horneophyton lignieri* spore (APICULIRETUSISPORA type). X 250.

Text-fig. 2. Camera lucida drawing of *H. lignieri* spore (EMPHANISPORITES DECORATUS). X 250.

Text-fig. 3-8. Camera lucida drawings of *Horneophyton lignieri* germinating spores. These have been arranged in order of possible developmental stages of gametophytes (cf. figs. 5-10). X 250.

and present in most of the specimes, slightly above the equatorial line (see figs. 1-2), contact area distinct. Exine ± 2μ thick excluding cones. Morphology of spores based on 15 specimens.

Dimensions: 44.1-71.4μ mean 56.6μ. Greatest diameter of 256 specimens was measured.

Locality: Scotish (?) Lower Old Red Sandstone, Rhynie chert, Aberdeenshire.

(b) *Comparison and discussion:*

The spores of *Horneophyton lignieri* are not attributable to any one form genus. Spores have been isolated from a single sporangium which can be referred to both *Apiculiretusispora* Streel (1967) and to *Emphanisporites* McGregor (1961).

Spores of the *Apiculiretusispora* type by far the most common and resemble quite closely to those described as *Apiculiretusispora* (*Cyclogranisporites*) *plicatus* (Allen) Streel (1967) apart from the absence of folds and slight difference in size. Although they are also quite similar in general appearence to those described as type "E" by Lang (1925) from the Middle Old Red Sandstone of Cromarty. Information regarding those is too scanty to enable any useful comparison to be made. The less abundant *Emphanisporites* type spores all lie at the lower end of the size range (44.1-56μ). The general form, size range and radially arranged pattern on the proximal surfaces is very similiar to that described for *Emphanisporites decoratus* Allen (1965).

Spores of more than one distinct type recognized within an individual sporangium is an interesting result of the work. So far as is known this has not been observed in any other early Devonian plants (see Banks 1968 & Edwards (1968).

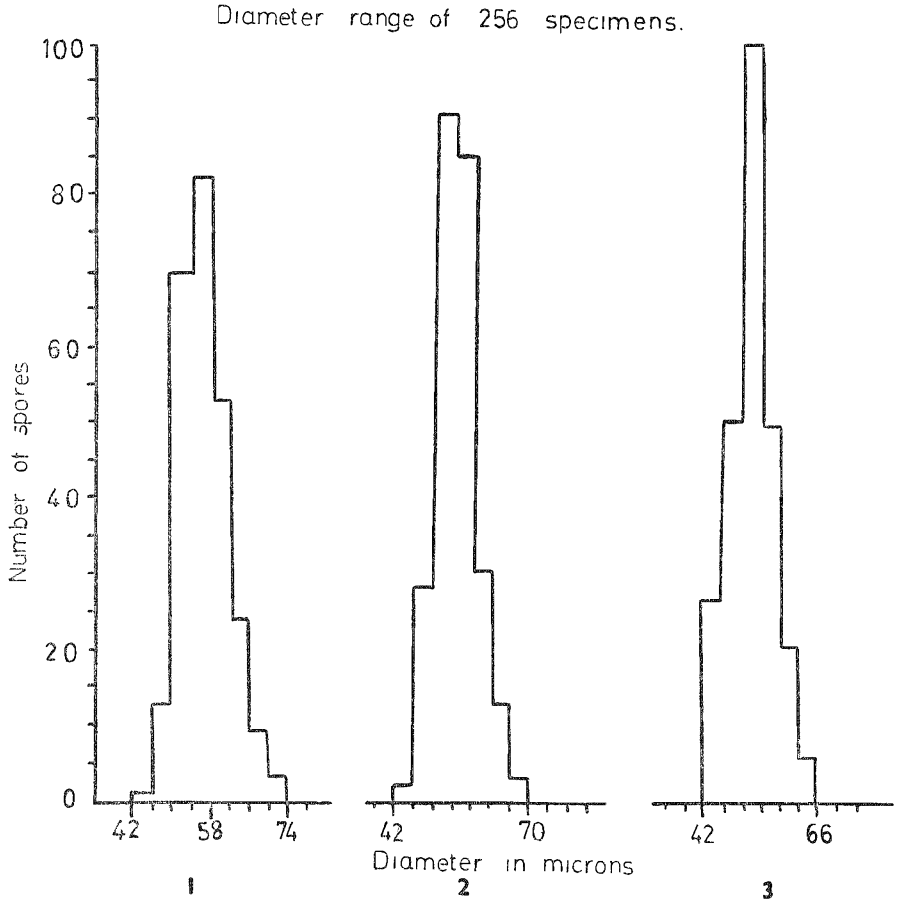
(c) *Size variation in relation to conditions of observation and preparation*

The spores of *Horneophyton lignieri* when shed tend to increase in size (Kidston & Lang, 1920). It is also known that chemical treatment can cause slight swelling (Richardson, 1960). That peel sections might also result in dimensional changes was also considered possible and some comparitive measurements relating to this were made. Results are summarized in Table I and Text-figs. 3-5.

The average size of spores in ground section of sporangia was used as basis for comparisons and variations expressed in percentages. Spores measured in peel sections of sporangia were all found to undergo apparent dimensional increase of 4.8% while isolated spores from sporangium showed an increase of size upto 8.9%. It is also known that average size of dispersed spores in the matrix of ground sections was found to be 10.9% greater than that of unshed spores in ground sections of sporangia of *Nothia aphylla* Lyon (Bhutta, 1969). It has been established that *Horneophyton lignieri* spores isolated from sporangia show a dimensional increase of 8.9%, it is not known whether dispersed spores in the matrix (if isolated), would again undergo a similar increase in size as is known in the case of *Nothia aphylla*. But if this were so, the total change could amount to 19.8% increase of size in relation to unshed spores.

Table I. **Size of spores belonging to a single sporangium of** *Horneophyton lignieri*.

Spores	No. of spores measured	Diameter Range μ	Mean Size μ	Size Increase in percentages
in ground section of sporangium ..	256	42.0 — 63.0	52.0 ± 0.27	—
in peel sections of sporangium ..	256	44.1 — 69.3	54.5 ± 0.28	+4.8
isolated from sporangium	256	44.1 — 71.4	56.6 ± 0.31	+8.9



Text-fig. 9. Spore size range
(1). Isolated from sporangium, (2). in peel sections of sporangium.
(3). in ground sections of sporangium,

(d) Germinated spores of *Horneophyton lignieri*

All the germinating spores which have been described and identified so far belong to the spore genera *Apiculiretusispora* Streel (1967). No germinating spores of the type *Emphanisporites decoratus* Allen have been found.

The total number of spores available for study was eleven and with reasonable certainty, be assigned to *Horneophyton*. Out of eleven spores, six germinating spores have been selected for description and illustration. Those which have been excluded were either duplicate or add no additional information.

Direct evidence in the form of photomicrograph are presented, although limitation inherent in this method of illustration resulting from lack of contrast and depth of focus will be readily apparent despite the use of "Montages". These have been supplemented, therefore, with Camera lucida drawings in which all visible details are included.

The germinated spores have been found in association with other plants and animal remains like fungal remains, *Nematoplexus rhyniensis*, *Algites* (*Palaeonitella*) *cranii*, *Rhynia gwynne-vaughani* (rhizome and stem), the crustacean *Lepidocaris* and *Lepidocaris*? egg cases.

*Germinating spore No.*1: Slide No. 79

Figure No. 5 Text-figure 3.

Description: Spore triradiate, spherical, dark brown, lateral oblique view, proximal face damaged, distal region ornamented with apiculae 2μ long, pointed. Spore size 56.7μ excluding apiculae. Commissure open forming a beak, no cellular structure visible. It might be the starting stage of germination. No curvaturae traceable due to dark colour of the spore.

Germinating spore No. 2: Slide No. LRS. 1

Figure No. 6 Text-figure 4

Description: Spore triradiate, estimated size 59μ, brown, lateral view, spherial, angle of dehiscence about 60°. Proximal surface smooth, distal ornamented with cones under 2μ long. Curvaturae perfectae distinct. Germinal out growth 84X63 μ, light coloured, bladder shaped, outer most layer of cells rectangular, central cells not clear but small in size and visible only under red light. Apical cells are slightly smaller than the rest of lateral cells. Germinating out growth in contact with the endosporium.

Germinating spore No. 3. Slide No. 251167A/3

Figure No. 7 Text-figure 5

Description: Spore triradiate, dark brown, spherical, estimated size 52μ, proximal face not clear, distal region ornamented with cones + 2μ,. Curvaturae not traceable. Germinal out growth 52μ wide at the base. Only two basal cells are visible which are narrow and elongated. Full length of out growth not measurable as it is faintly developed towards the apex.

Germinating spore No. 4. Slide No. 251167A/3

Figure No. 8 Text-figure 6

Description: Spore triradiate, dark brown, distal face ornamented with cones ±2μ. Estimated spore size 54.6μ. Germinal out growth distinct, filamentous, 3-4 cells wide, cells are elongated and most of them shrunken. Out growth 20μ wide at the base and 160μ in length, pointed towards the apex. Fungal hypha are attached to the main filamentous out growth visible both in photomicrgrpah and text-figure.

Germinating spore No. 5. Slide No. 79

Figure No. 9 Text-figure 7

Description: Spore triradiate, dark brown, spherical, estimated size 70μ, apiculate and cones ±2μ long. Germinal out growth light coloured and distinct, 74μ

wide at the base and 196μ in height. Apical cells on one side are two in number forming a notch (N). Below the apical cells two blackish depositions of unknown significance are present.

Germinating spore No. 6. Slide No. LRS 12

Figure No. 10 Text-figure 8

Description: Spore triradiate, dark brown, spherical, estimated size 70μ, apiculate, apiculae ± 2μ long. Germinal out growth wide at the base, curve around and narrows towards the apex but apical cells broadend forming a 'corona" shaped structure. 'Coronal' cells are relatively bigger and three in number on one side and some diffusely formed cells are also present to the extremities of the apex. Estimated length of out growth 300μ, curving region 87μ wide, apical 'coronal' region 42μ wide.

Comments

Germinated spores have been identified on the basis of information available from isolated spores of the plant. It is interesting to mention that no other spore ornamented with apiculae has been discovered assignable to any other Rhynie vascular plant (Bhutta, 1969). Although the probable developmental stages have been recognized in *Horneophyton lignieri* germinating spores, no indication of sexual reproductive organs have been found nor rhizoids seen. Nevertheless, there can be little doubt that these delicate structures represent young gametophytes and their association with both Charophyte and Crustacean remains suggests that germination may have taken place under very wet conditions.

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