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RESEARCH ARTICLE

Microscopic Analysis of Epidermal and Vascular Structures in *Alysicarpus naikianus* Pokle: Anatomical Adaptations for Environmental Resilience

Shantanu Y. Chavan[1], & Rupali R. Taur[2]

[1]Dept of Botany, Sant Dnyaneshwar Mahavidyalaya Soegaon, Dist. Chhatrapati Sambhajinagar 341120 (MS) India.

[2]Dept of Biosciences and Technology, MGM University, Chhatrapati Sambhajinagar 431003 (MS) India.

**Corresponding Author*: alysicarpus@gmail.com

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ABSTRACT

Alysicarpus naikianus Pokle is an endemic species of the genus *Alysicarpus* in the subfamily Papilionoideae, tribe Desmodieae, of the Leguminosae family. Described in 1999, this species was initially known from limited collections at three localities in Maharashtra, India. Recent floristic surveys, particularly in the hilly areas of the Eastern Ghats of Tamil Nadu and western India, have revealed additional populations, expanding its known distribution. The species exhibits morphological characteristics consistent with other members of its genus, including distinctive Anatomy of leaf, stem, root, vessel's structure and epidermal features, which were studied using light microscopy. The findings indicate a higher level of diversity and a broader ecological range than previously documented. As a result, *A. naikianus* is now recognized as a significant species within the *Alysicarpus* genus, highlighting the need for further exploration and conservation efforts in its native habitats.

Keywords: *Alysicarpus naikianus* Pokle, Anatomy, Dermatology, Vessels.

INTRODUCTION

The genus *Alysicarpus* Necker ex Desvaux (1813: 120) belongs to the subfamily Papilionoideae, tribe Desmodieae of Leguminosae. This genus comprises approximately 34 species and 20 infraspecific taxa, widely distributed across the tropical and subtropical regions of Africa, Asia, and Australia, with the highest diversity found in India (Pedley 2001, Adema 2003, Lewis 2005, Huang and Ohashi 2010, Hassemer et al. 2017, Leeratiwong et al. 2017, Pokle 2017). In India alone, about 17 species and 9 infraspecific taxa of *Alysicarpus* are present, of which seven species and eight infraspecific taxa are endemic (Sanjappa 1992, Pokle 2002, Dhabe 2013, Chavan 2013, Singh et al. 2015, Gholami et al. 2017, Pokle 2017).

Alysicarpus naikianus Pokle is an endemic species described in 1999, and until recently was only known from a few collections from three localities: Appachiwadi, Kakti, and Shivaji University Kolhapur campus, Maharashtra (Chavan 2012). During floristic explorations in various hilly areas of the Eastern Ghats of Tamil Nadu, a few specimens belonging to the genus *Alysicarpus* were collected from the Yercaud hills (Parthipan 2013). In recent surveys, several additional populations were found in western India, in addition to the type localities. These populations are now known to be chiefly distributed along the eastern escarpments of the Western Ghats and the coastal plains of Kerala, Karnataka, Goa, and Maharashtra.

MATERIAL & METHODS

Anatomical Studies

The anatomical studies of *Alysicarpus naikianus* Pokle. were conducted on the roots, stems, and leaves. The investigations were based on transverse sections (T.S.) obtained by hand sectioning using sharp razor blades. Permanent slides of these sections were prepared using different grades of alcohol and xylol, with safranin and light green stains. Observations were made under light microscopes and photographed with a Sony Cyber Shot DSC-S950 digital camera.

Plant Material

The plant materials used for these investigations were preserved in 4% formaldehyde solution. Collections of *A. gamblei* were made from a single locality on the Badami Plateau, Karnataka. Samples were taken from fully expanded, sun-exposed leaves.

Preparation for Light Microscopy

Material for light microscopy was boiled in water for 10 minutes and then transferred to Jeffrey's solution for maceration (Zheng, 1978) for one hour. After maceration, the adaxial and abaxial epidermal tissues were separated from other tissues, washed with distilled water, and stained in a 1% safranin solution before mounting in Canada balsam.

Consistency and Quantitative Analysis

To ensure consistency in the examination of epidermal structures under the light microscope (Labomed Vision 2000), at least ten slides were prepared from a single leaf or from different leaves of each form. For statistical analysis, a unit area of 1 mm^2 was measured using an ocular and stage micrometer. Epidermal cells and stomata were counted using a micrometer eyepiece. The range, mean, standard deviation, and coefficient of variation were determined for all taxa. The stomatal index was calculated using the formula by Salisbury (1927):

Stomatal Index (I) = $[S \div (E + S)] \times 100$ where S= is the number of stomata per unit area, and E= is the number of epidermal cells in the same unit area. Photomicrographs were taken using a Labomed Vision 2000 microscope with an attached camera.

RESULTS

T.S. of Root

The outermost layer epidermis surrounded by the thin walled epiblema, epidermis single layered, the epidermal cells rectangular, ca 5-10 × 3-6 µm. Below the epidermis 2-4 layered, composed oval, circular, rectangular compactly arranged parenchymatous cells ca 6-12 × 3-6 µm. Cortex 3-6 layered; the cortical cells are oval, circular, isodiametric parenchymatous cells ca 5-10 × 3.5-7.5 µm. Phloem 2-4 layered, polygonal, squarish ca 3-5 × 2-3 µm. Cambium 1-2 layered, horizontally elongated cells ca 3-5 × 1-2 µm. The metaxylem 3-5 layered, circular, oval, hexagonal ca 8-12 × 7-10 µm. Protoxylem circular, hexagonal, oval ca 2.5-7 × 2-4.5 µm. Medullary rays radially arranged.

T.S. of Stem

The epidermis single layered, composed of elongated, barrel shaped cells, ca 10-15 × 7.5-12.5 µm. The cortex is made up of 1-2 layers of parenchymatous cells, are hexagonal in shape ca 7.5-17.5 × 6.25-12.5 µm. Below the cortex single layer of pericycle is present, it is made up

of barrel shape, elongated compactly arranged cells, ca 20-27.5 × 10-15 µm. Pericycle fallowed by 1-3 layers of phloem, it is composed of circular to hexagonal cells, ca 6.25-11.25 × 10-15 µm. The vascular bundle sheath conjoint, bicollateral. The external phloem, 2-4 layered, rectangular, tangentially elongated cell, ca 3.75-7.5 × 2.5-6 µm. Metaxylem 2-4 layered, circular to hexagonal cells surrounded by xylem parechymatous cell, ca 15-27.5 × 20-30 µm. Protoxylem circular to hexagonal, ca 10-15 × 12.5-22.5 µm. Primary medullary rays present. Intraxilary phloem 2-3 layered. Below the intraxilary phloem the lysigenous cavity is present, surrounded by intraxilary phloem. Pith parenchymatous, occupying a large part of the stem. Cells thin walled, oval, rounded, hexagonal, consisting irregular crystals.

T. S. of Leaf

The transverse section of the leaf showed typical dorsiventral structure. The epidermis of both the surfaces single layered, covered with thin cuticle and leaf papillae is present. The upper epidermis composed of rectangular or squarish cells. The upper epidermis cell range from 20-37.5 × 8.75-13.75 µm. The lower epidermal cell oval or rectangular in shape, the lower epidermal cell ranges from 7.5-22.5 × 5-17.5 µm. Epidermal cells at the midrib region are smaller than the lamina region.

Mesophyll is diffrentiated in to two layered viz. Palisade tissue and Spongy tissue. Palisade tissue in 2-3 layered, composed of vertically elongated, which are quite compact to each other the range 15-30 × 6.25-10 µm. Spongy mesophyll circular, oval, loosely arranged cells ranges 7.5-17.5 × 8.75-20 µm, with intercellular spaces on the lower side. Some mesophyll cell showed prismatic, irregular crystals.

At the midrib region, epidermis fallowed by single layered palisade parenchyma. Parenchymatous cells 2-4 layered, circular, hexagonal in shape ca 17.5-35 × 10-37.5 µm. Inner cortex 2-4 layered composed of polygonal parenchymatous cells. Bundle sheath circular, oval or compactly arranged ca 7.5-15 × 8.75-13.75 µm. The main vascular bundle surrounded by Sclerenchyma 2-5 layered, with irregular, oval cells ca 5-10 × 7.5-12.5 µm. Phloem 3-5 layered, compactly arranged with irregular or circular cells ca 5-15 × 5-10 µm. Xylem 2-4 layered, squarish, hexagonal cells ca 7.5-25 x 6.25-20 µm. The vascular bundle is conjoint, collateral closed and surrounded by thin walled parenchymatous bundle sheath.

Dermatology

Upper epidermis- Two types of stomata observed i.e paracytic (rubiaceous), anomocytic (ranunculaceous), amphistomatic, range of pore length 7.5-12.5 µm, average 9 µm; guard cells range 12.5-20 × 2.5-6.25 µm, average 15.25-3.88 µm; epidermal cells hexagonal, range 22.5-35 × 15-42.5 µm, average 29 × 22.25 µm in dimension (length & width); anticlinal walls straight. The stomatal number 15 and stomatal index is 16.30. (Plate -1 Fig.5a).

Lower epidermis- Stomata paracytic, range pore length 7.5-12.5 µm, average 9.75 µm; guard cells range 15-22.5 × 6.25-7.5µm, average 18.75 × 6.50 µm; epidermal cells irregular, range 22.5-47.5 × 15-30 µm, average 33 x 20 µm in dimension (length & width); anticlinal walls curved. The stomatal number 15 and stomatal index is 9.33.

Vessels

Vessel elements not much longer, end wall horizontal, with simple perforation, pits are circular, oval, alternate, beak presents. The range of vessel elements is ca 170-470 × 15-40 µm, mean ca 293 × 29 µm, the range of vessel index is 42.5-240 µm.

DISCUSSION

The anatomical study of *Alysicarpus naikianus* reveals several distinct features in the root, stem, leaf, and vessels that highlight the unique structural adaptations of the species. These findings provide valuable insights into its functional morphology and may have taxonomic significance in distinguishing this variety from closely related species.

Root Anatomy

The transverse section of the root shows a well-defined epidermal layer, which is compact and rectangular in shape, serving as the outermost protective layer. The presence of multiple layers of parenchymatous cells beneath the epidermis suggests a potential role in storage and transport. The cortex consists of isodiametric parenchymatous cells, which contribute to the mechanical support and nutrient storage in the root system. Vascular tissues, including phloem and metaxylem, exhibit characteristic polygonal, squarish, and hexagonal shapes. The arrangement of medullary rays in the radial direction enhances the radial conduction of water and nutrients, which is essential for the plant's survival in varying environmental conditions.

Stem Anatomy

The stem section reveals a single-layered epidermis made up of elongated, barrel-shaped cells, which is typical in many herbaceous plants. Below the epidermis, the cortical parenchyma supports basic physiological functions, while the pericycle and vascular bundles play critical roles in the conduction of water, minerals, and organic substances. The conjoint and bicollateral nature of the vascular bundle indicates a well-developed system for efficient transport. Notably, the presence of both external and internal phloem and the pith occupying a large part of the stem point to a well-adapted system for transport and storage of nutrients. The lysigenous cavity seen in the stem is a remarkable feature, possibly contributing to gas exchange or the storage of secretory substances.

Leaf Anatomy

The leaf anatomy of *A. naikianus* displays a typical dorsiventral structure, characterized by a well-developed upper and lower epidermis. The mesophyll differentiation into palisade and spongy tissues reflects the plant's adaptation to efficient photosynthesis. The compact arrangement of palisade cells aids in maximizing light capture, while the loosely arranged spongy mesophyll allows for gas exchange. The irregular crystals in the mesophyll cells may play a role in defense against herbivores or in other physiological processes. The well-structured vascular bundle, surrounded by sclerenchyma, provides mechanical support and protection to the conducting tissues.

Dermatological Features

The presence of both paracytic and anomocytic stomata on the leaf surfaces, along with amphistomatic stomatal distribution, suggests that *A. naikianus* is well-adapted to varying environmental conditions. The hexagonal shape of epidermal cells and the anticlinal walls indicate structural stability and protection. The stomatal indices of 16.30 (upper epidermis) and 9.33 (lower epidermis) indicate the leaf's balance between gas exchange and water retention, essential for survival in different habitats.

Vessel Anatomy

The vessel elements in *A. naikianus* are relatively short, with a horizontal end wall and simple perforation, which is characteristic of plants in water-stressed environments. The range of vessel element lengths (170–470 µm) and widths (15–40 µm) demonstrates the variation in xylem architecture, which may enhance the plant's ability to transport water efficiently. The circular and alternate pits within the vessels facilitate water conduction, while the presence of beaks suggests specialized structural modifications for efficient water movement.

The variation in vessel index (42.5–240 µm) reflects a wide range of vessel sizes, indicating adaptations to different water availability conditions. This variability may allow the plant to regulate water transport depending on environmental stressors, such as drought or flooding.

Discussion of the Given Tables

The data presented in Table 1 and Table 2 provide detailed insights into the epidermal characteristics and vessel elements of *Alysicarpus naikianus*. The analysis highlights variability in epidermal cell dimensions, stomatal types, guard cell sizes, and vessel element properties, all of which are crucial for understanding the plant's adaptive strategies and functional morphology.

Epidermal Characteristics (Table 1)

Table 1 summarizes the epidermal cell, stomata, and guard cell dimensions of *A. naikianus* under a light microscope. The upper epidermal cells show a range of lengths from 22.5 µm to 35 µm and widths from 15 µm to 42.5 µm, with an average length of 29.00 µm and width of 22.25 µm. The lower epidermal cells are slightly larger, with a range of 22.5 µm to 47.5 µm in length and 15 µm to 30 µm in width, indicating a more varied and irregular structure compared to the upper epidermis. This variability in epidermal cell size is consistent with their functional roles in protection, water retention, and photosynthesis regulation.

The stomatal characteristics further emphasize the plant's adaptive mechanisms. Two types of stomata are observed: paracytic (rubiaceous) and anomocytic (ranunculaceous). The stomatal pores on the upper epidermis range from 7.5 µm to 12.5 µm in length, with a mean of 9.00 µm, while the lower epidermal pores range from 7.5 µm to 12.5 µm with a mean of 9.75 µm. The slight increase in pore size on the lower epidermis suggests a higher potential for gas exchange in this region, as seen in many amphistomatic plants, which have stomata on both leaf surfaces to optimize gas exchange under varying conditions.

The guard cells on the upper epidermis range from 12.5 µm to 20 µm in length and 2.5 µm to 6.25 µm in width, with an average of 15.25 µm by 3.88 µm. The lower

epidermal guard cells are larger, ranging from 15 µm to 22.5 µm in length and 6.25 µm to 7.5 µm in width, with an average of 18.75 µm by 6.50 µm. The larger size of the lower epidermal guard cells may facilitate better control over stomatal aperture, which is essential in regulating transpiration and water loss, especially in response to environmental stresses.

The coefficient of variation (CV) values for epidermal cell dimensions are relatively high, particularly in the lower epidermis, with CV values of 35.31% for length and 26.35% for width, indicating significant variability in the size and shape of these cells. This morphological diversity may reflect adaptations to varying environmental conditions, where different sizes of epidermal cells and stomata help the plant regulate water balance, gas exchange, and protection against environmental stress.

Vessel Elements (Table 2)

Table 2 provides measurements of the vessel elements, highlighting their structural dimensions and functional properties. The vessel elements range in length from 170 µm to 470 µm, with a mean of 293 µm, while the width ranges from 15 µm to 40 µm, with a mean width of 29 µm. These vessels are relatively short, but with a considerable variation in size, as indicated by the high standard deviation (SD = 97.30 for length, 9.07 for width), and the coefficient of variation for vessel length is 33.21%. This variability in vessel length and width likely reflects the plant's ability to transport water efficiently under different environmental conditions.

The vessel index, which is a measure of the length-to-width ratio of vessel elements, ranges from 42.5 to 240, with a mean of 118.37. A higher vessel index generally indicates vessels that are long and narrow, which are advantageous in water-limited environments as they reduce the risk of embolism (air blockages in the xylem). The variation in vessel index (CV = 58.86%) suggests that *A. naikianus* has a diverse xylem structure, which may provide flexibility in coping with fluctuating water availability. This variability also allows the plant to maintain efficient water transport under stress, supporting its survival in different ecological conditions.

CONCLUSION

The detailed anatomical investigation of *Alysicarpus naikianus* highlights its structural adaptations, particularly in the root, stem, leaf, and vessels. The species displays features such as a well-defined epidermis, robust vascular system, specialized stomatal distribution, and variable vessel elements, which together suggest its ability to thrive in different ecological conditions. These anatomical traits provide a basis for further taxonomic classification and contribute to our understanding of the physiological adaptations of the species. Future studies could focus on correlating these structural features with the ecological and environmental factors influencing the plant's distribution and growth patterns.

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Table No. 1. Characters of Leaf Epidermis of *Alysicarpus naikianus* Pokle. Taxa Under Light Microscope.

Sr. No	Epidermal Cell				Stoma		Guard Cell			
	Upper µm		Lower µm		Upper µm	Lower µm	Upper µm		Lower µm	
	Length µm	Width µm	Length µm	Width µm			Length µm	Width µm	Length µm	Width µm
1	35	22.5	27.5	15	8.75	7.5	15	3.75	17.5	6.25
2	30	17.5	35	20	10	8.75	17.5	5	20	6.25
3	32.5	20	40	22.5	12.5	7.5	20	6.25	22.5	7.5
4	22.5	20	30	17.5	10	7.5	15	5	15	6.25
5	22.5	42.5	27.5	20	7.5	10	15	3.75	17.5	5
6	32.5	15	42.5	27.5	8.75	10	12.5	2.5	20	6.25
7	27.5	25	47.5	30	8.75	12.5	15	3.75	20	7.5
8	30	17.5	25	15	7.5	12.5	12.5	2.5	17.5	6.25
9	27.5	17.5	22.5	15	8.75	12.5	15	3.75	17.5	6.25
10	30	25	32.5	17.5	7.5	8.75	15	2.5	20	7.5
Range	22.5-35	15-42.5	22.5-47.5	15-30	7.5-12.5	7.5-12.5	12.5-20	2.5-6.25	15-22.5	6.25-7.5
Mean	29.00	22.25	33.00	20.00	9.00	9.75	15.25	3.88	18.75	6.50
SD	4.11	7.85	8.14	5.27	1.53	2.10	2.18	1.24	2.12	0.79
CV	14.19	35.31	24.69	26.35	17.07	21.62	14.35	32.07	11.33	12.16

Table No. 2. Microscopic Study of Vessels Elements.

Pitted			
Sr No	Length (µm)	Width (µm)	Index
1	170	40	42.5
2	200	35	57.143
3	350	15	233.333
4	470	35	134.286
5	170	35	48.571
6	280	30	93.333
7	360	15	240
8	250	20	125
9	360	35	102.857
10	320	30	106.667
Range	170-470	15-40	42.5-240
Mean	293	29	118.369
(SD)	97.3025	9.0676	69.6730
(CV)	33.2090	31.2677	58.8609

Plate I

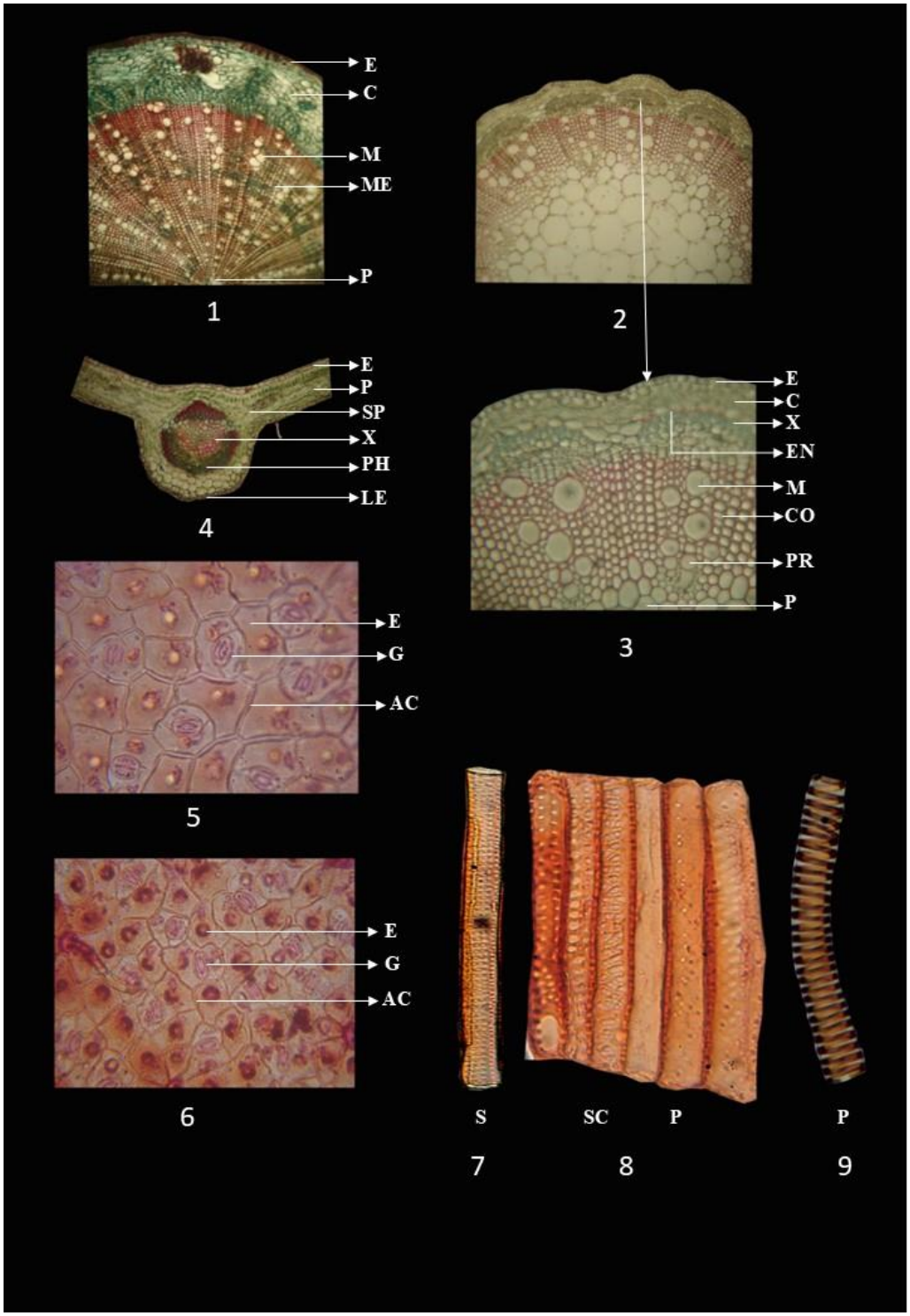

Fig.1. E-Epidermis, C-Cortex, P- Protoxylem, M-Metaxylem, P-Pith. **Fig.2.** T.S. of Stem. **Fig.3**. E-Epidermis, C-Cortex, X-Xylem, EN. Endodermis, M. Metaxylem, CO. Cortex, PC- Pericycle Cell, PH-Phloem, C-Cortex, MR-Medullary Rays, MX- Metaxylem, P-Pith. **Fig.4.** E-Upper Epidermis, PP-Palisade Parenchyma, SP-Spongy parenchyma, X-Xylem, PH. Phloem, LE-Lower epidermis. **Fig.5.** EC-Epidermal Cell, GC. Guard Cell, AW-Anticlinal Wall. **Fig. 6.** EC-Epidermal Cell, GC. Guard Cell, AW-Anticlinal Wall, F. B-Beak. **Fig.7.** Scalariform, **Fig**.8. Scalariform, P-Pitted. **Fig.9**. G-Spiral.