

**CARPOPHORE SHAPES IN *TRAMETES VERSICOLOR* (L.) LOYD  
(POLYPORACEAE) USING ELLIPTIC FOURIER TRANSFORMS AND  
PRINCIPAL COMPONENT ANALYSIS**

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**Abstract:**

*Dimension and shape analysis is fundamental scientific approach in many biological investigations. Trametes versicolor is one of widespread xilobionts which colonizes decomposing wood as well as live wooden substrates inducing white rot. Substrate colonization is established by means of basidiospores, accordingly their dispersion is of outmost importance. The shape of carpophores determines dispersion success and accordingly niche occupancy by T. versicolor. Based on scanned and digitally analyzed images of T. versicolor carpophores, shape quantitative description was performed using Elliptic Fourier Transforms and Principal Component analysis which summarizes Fourier descriptors. PC scores were employed as quantitative characteristics for average shape reconstruction through Inverse Elliptic transformation. Continuous shape variation was evaluated through symmetrical and asymmetrical coefficients in more appropriate way than in classical morphometric analysis based on measurement descriptors.*

**Key words:** Elliptic Fourier Transform, Principal Component analysis, *Trametes versicolor*, carpophore shapes, ANOVA, simple regression

**INTRODUCTION**

Dead wood is a key resource in forest ecosystems (Tews et al., 2004; Komonen & Kouki, 2005) and harbors complex communities of diverse organisms (Jonsson et al., 2005). Wood degrading fungi use wood as resource and habitat being the most important group of organisms involved in decomposition and central nodes of wood related trophic webs.

During successional stages of stump decomposition biochemical differences between tree species decrease in importance for degrading communities assemblage and site conditions become more important in featuring these communities (Tyler, 1992). Wood anatomy and the biochemistry of secondary metabolites select degradative fungi during the first stages of decomposition (Hintikka, 1982). Xylobiotic fungi become habitat and resource for many other groups of organisms such as nematodes and insects.

*Trametes versicolor* (L.) Lloyd (Polyporaceae) is a white rot fungus forming annual bracket like basidiomata which start to develop early in the spring and reach complete development during the autumn. Due to woody texture, basidiomata can remain attached to substrate for two years when they function as habitat and food resource for beetles from Fam. Ciidae, other invertebrates and degradative micromycetes. *T. versicolor* is a polyphagous basidiomycete being associated with many broadleaved and coniferous trees and shrubs as well as wooden artifacts such as wooden fences, poles or buildings' wooden structures. It was discovered also that this species displays a large array of healing properties in chronic fatigue syndrome, autoimmune diseases, pulmonary and digestive diseases, improves general state in colon and liver cancer, in psoriasis, lowers cholesterol level, being one the most largely commercialized alternative cures in the world (Yanagi & Minoru, 1977; Hobbs, 2004). It is utilized at industrial scale for laccase production, for

waste water detoxification, as bleaching agent in pulp and textile industry (Cauto &Toca-Herrera, 2007).

Morphological variation in size and shape has physiological, ecological and taxonomic significance (Somers, 1989). One of central morphometric topics is the separation of dimensional and shape descriptors (Rohlf & Bookstein, 1987). Shape is considered a better morphological descriptor because dimension varies at ontogenetic as well as phylogenetic scale (Sunberg, 1989). At empirical level, shape is the geometry of a configuration (Marcus et al., 1996).

An objective quantitative shape evaluation is provided by Fourier coefficients obtained through Fourier Analysis. A procedure based on Elliptic Fourier descriptors is commonly used to analyze the shape of plant organs (Kuhl & Giardina, 1982). Principal Component Analysis is applied on Fourier coefficients considered as distinct variables to summarize the data (Rohlf & Archie, 1984). The elliptic Fourier descriptors can separate any type of shape with a closed two dimensional contour and were employed for the analysis in various plants species such as *Betula* leaves (White et al., 1988), *Begonia* leaves (McLellan, 1993), buckwheat kernels (Ohsawa et al., 1998), soybean leaves (Furuta et al, 1995), petal shape in *Primula sieboldii* (Yoshioka et al. 2004). Elliptic Fourier Analysis supplies the coefficients of trigonometric functions that reproduce as closely as possible a sample curve. As more terms (harmonics) are added to the function, the fit of the sample curve becomes better. As particular EFA coefficient values constrain the function to follow a closed outline, they provide descriptors of the outline that can be compared to corresponding coefficients from other outlines: Principal Component Analysis provides the means to compare the coefficients (McLellans & Endler, 1998) being a linear transformation that minimizes the covariance between transformed features along the coordinate axes and maximizes the variance, so features (Fourier coefficient in our case) become perfectly uncorrelated. Iwata and Ukay (2002) developed a software package SHAPE for quantitative evaluation of biological shapes on the basis of Elliptic Fourier Analysis coupled with Principal Component Analysis.

The primary focus of the present research was to characterize the shape of *Trametes versicolor* basidiomata as conditional factor for successful spore dispersal. Colonization of new substrata is mainly by basidiospores, consequently shape and position are important for discharge in the air flow and further spore fate in terms of dispersal distances. Classical morphometric analysis based on comparisons between samples statistics (ANOVA performed on areas, perimeters, elongations) and multivariate analysis of metric descriptors using Principal Components analysis were also performed.

Shape and dimensions are important organism descriptors which variations are physiologically and ecologically significant. Also aspects concerning interspecific relationships of *T. versicolor* with other species inhabiting same substrate or using basidiomata as habitat and food resource are considered.

## MATERIALS AND METHODS

Samples of 30 carpophores randomly collected from different substrates in three locations were photographed using Konica-Minolta camera. Substrata were represented by stumps of *Berberis vulgaris* (garden city of Cluj-Napoca, block Grigorescu), of *Prunus sp.* (recreational forest Hoia, Cluj-Napoca), of *Prunus avium* (Făget recreational forest, Cluj-Napoca) and samples (90 carpophores) were collected in the spring of 2008. Images were processed using the soft Xara Xtreme Pro for further utilization in Shape soft which performs Elliptic Fourier Analysis and Principal Component Analysis (Iwata & Ukay, 2002) on processed images. Image Tool soft was employed for dimensional morphometric measurements on images (areas, perimeters, lengths, widths and elongations of basidiomata)

also principal Component Analysis was performed on these measurements. Additional measurements on lengths and width using digital caliper of 510 basidiomata, collected from different locations (Cluj-Napoca recreational forests, Oradea recreational forests, Bulz rural area in Bihor county, Cheile Nerei National Park, Cheile-Carasului Reserve, Sangiorz-Bai town in Bistrita-Nasaud county, Borcut Forest, Bratca valley in Bihor county) and 10 woody species (*Carpinus betulus*, *Malus sylvestris*, *Fagus sylvatica*, *Quercus rubra*, *Tilia cordata*, *Fraxinus excelsior*, *Crataegus monogyna*, *Picea abies*, *Salix sp*, *Alnus glutinosa* and also from a wooden fence) were used to perform Linear Regression Analysis, ANOVA (on original data and log transformed) using KyPlot soft.

*Elliptic Fourier Analysis* is based on the geometric representation of each harmonic pair of order  $n$  that could be represented as an ellipse. Each ellipse is represented by four parameters, the elliptic descriptors related to major axis, minor axis, angle of rotation corresponding to length axis orientation of the ellipse and the angle of phase corresponding to the position of the first point on the ellipse.

The outlines are reconstructed, step by step using an increasing number of harmonics, carried point by point. The resulting elliptic descriptors can be subjected to statistical analysis as variables describing the shape. Fourier coefficients can be treated as independent variables because they satisfy the condition of orthogonality. This permits the assessment of the proportional contribution of each component to the overall variability between specified groups. The property of orthogonality allows the partition of size and shape components (Lestrel et al., 1977) which means that it is possible to separately analyze the contribution of each harmonic to the total form. (form=size+shape)

The Fourier coefficients (descriptors) are summarized by means of Principal Component Analysis and PC scores are used to characterize the shape of the objects subjected to analysis. Effect of each PC is visualized by recalculation of inverse Fourier descriptors (Iwata & Ukay, 2002).

In the present study, carpophores of *T. versicolor* were photographed with Konica –Minolta camera and photographs were processed using the software Xara Xtreme Pro then being imported in SHAPE software. The contours are chain-coded by the software and represented as a sequence of  $x$  and  $y$  coordinates. Each point coordinates are described by the equations:

$$x_p = \sum_{i=1}^p \Delta x_i \quad y_p = \sum_{i=1}^p \Delta y_i$$

The elliptic Fourier expressions of the coordinates are:

$$x_p = A_0 + \sum_{n=1}^{\infty} \left( a_n \cdot \cos \frac{2n\pi p}{T} + b_n \cdot \sin \frac{2n\pi p}{T} \right) \quad y_p = C_0 + \sum_{n=1}^{\infty} \left( c_n \cdot \cos \frac{2n\pi p}{T} + d_n \cdot \sin \frac{2n\pi p}{T} \right)$$

The elliptic Fourier coefficients of the  $n$ -th harmonic are given by:

$$a_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^k \frac{\Delta x_p}{\Delta t_p} \left( \cos \frac{2n\pi p}{T} - \cos \frac{2n\pi p-1}{T} \right) \quad b_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^k \frac{\Delta x_p}{\Delta t_p} \left( \sin \frac{2n\pi p}{T} - \sin \frac{2n\pi p-1}{T} \right)$$

$$c_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^k \frac{\Delta y_p}{\Delta t_p} \left( \cos \frac{2n\pi p}{T} - \cos \frac{2n\pi p-1}{T} \right) \quad d_n = \frac{T}{2n^2\pi^2} \sum_{p=1}^k \frac{\Delta y_p}{\Delta t_p} \left( \sin \frac{2n\pi p}{T} - \sin \frac{2n\pi p-1}{T} \right)$$

It is worth to specify that sine terms describe the asymmetry and the cosine terms the symmetry (Lestrel et al., 1977).

According to the software, the shape was approximated by the first 20 harmonics. Because the coefficients are not invariant with respect to position, size, rotation, shift and starting point those were standardized by the program as recommended by Kuhl and Giardina, (1982).

The 80 resulted coefficients were classified in two groups related to symmetrical (a and d) and asymmetrical (b and c) variations with respect to central axis of each carpophore. The outline reconstructed on the basis of symmetrical coefficients is completely symmetric while the outline reconstructed on the basis of asymmetrical coefficients shows asymmetrical features of the carpophores.

Coefficients were subsequently used in Principal Component Analysis which summarized the data, based on covariance matrix. Inverse Fourier transform was employed to visualize the outlines described by PC scores. Original shape can be fully recovered from its Fourier Transform using Inverse Fourier Transform. Principal Component analysis is a multivariate method which summarizes variance structure among numerous variables by reducing them to a smaller number of uncorrelated variables named components (Shlens, 2005).

*Simple Linear Regression* was performed on dimensional data in order to establish the type of relationship between dimensional variables: allometric, either isometric. The variables were: area, perimeter, length, width of the carpophores as typical dimensional variables and elongation or aspect which is the length/width ratio, a typical dimensionless shape descriptor (Chen & Nelson, 2004). Somers (1989) showed that if the slope of the regression line is close to 1 on log-log plots, the relationship between tested dimensional variables is isometric, meaning bivariate change in size with no concomitant change in shape. If slopes differ from 1, bivariate changes are correlated with changes in size (allometric change). Log transformed measurement data (510 carpophores were measured with a digital caliper for lengths and widths) were employed and KyPlot software was used to perform the regression. Log transformation is necessary to ensure the independence of data from measurement units, keeping scale information unmodified (Marcus et al., 1996)

*Extraction of maximized variability in measurement data:* The photographed carpophores were assessed for dimensional data such as: area, perimeter, major axis and minor axis using as software Image Tool. Principal Component Analysis was performed on correlation matrix based on measurement data using the software KyPlot.

*Dimensional variability* of the carpophores sampled from different substrata was assessed using one-way ANOVA using measured on photographs dimensional variables (area, perimeter, length, width and elongation).

## RESULTS

Elliptic Fourier Analysis coupled to Principal Component Analysis resulted in the generation of the average carpophore's shape. The results on eigenvalues, symmetrical and asymmetrical coefficients, proportion of variability accounted for each Principal component are summarized in table I.

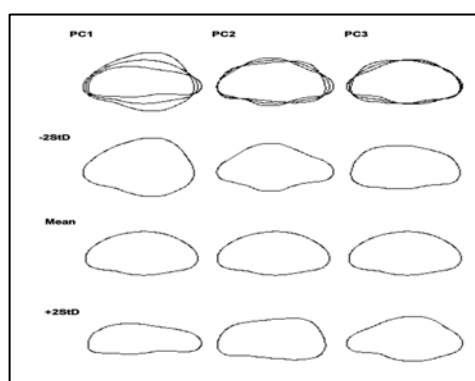
**Table 1**

The eigenvalues and principal Components, their contribution to total variability corresponding to symmetrical and asymmetrical Fourier coefficients in *Trametes versicolor* carpophores

Principal Component	Symmetrical Fourier Coefficients			Asymmetrical Fourier Coefficients		
	Eigenvalues (10 <sup>-4</sup> )	Proportions %	Cumulative proportions %	eigenvalues (10 <sup>-4</sup> )	Proportions	Cumulative proportions
<i>Trametes versicolor</i> – Făget-Valea Morii						
1	1.08E-02	77.82	77.82	4.26E-03	65.59	65.39
2	1.33E-03	9.56	87.39	1.08E-03	16.57	82.16
3	9.22E-04	6.63	94.02	3.08E-04	4.74	86.90
4				2.63E-03	4.05	90.95
5				1.84E-04	2.83	93.79
<i>Trametes versicolor</i> –Hoia forest						
1	1.00E-02	70.09	70.09	2.39E-03	48.43	48.43
2	2.47E-03	17.26	87.35	8.78E-04	17.78	66.22
3	5.96E-04	4.17	91.53	6.71E-04	13.58	79.80
4	3.88E-04	2.71	94.24	4.91E-04	9.93	89.74
5				1.64E-04	3.31	93.06
<i>Trametes versicolor</i> – block Grigorescu, Cluj-Napoca						
1	1.39E-02	71.20	71.20	3.04E-03	42.21	42.21
2	3.10E-03	15.87	87.07	2.12E-03	29.45	71.67
3	8.01E-04	4.10	91.18	6.89E-04	9.57	81.24
4	5.04E-04	2.58	93.77	3.93E-04	5.45	86.70
5				2.29E-04	3.17	89.87
6				2.01E-04	2.79	92.66

First three or four PC's were selected as cumulating most of variability (over 90%) for symmetrical coefficients and up to 6 PC in the case of asymmetrical coefficients.

According to literature data (Iwata et al., 2002; Yoshioka et al., 2005) and our evaluation, first principal component PC1 corresponds in the case of symmetrical coefficients to the aspects or the ration between length and width of the carpophores and accounts for 77% of explained variance at Făget, 70.09% at Hoia and 71% at Grigorescu, while PC2 corresponds to the centroid position and account for 9.56% of explained variance at Făget, 17.26% at Hoia and 15.87% at Grigorescu.



**Fig. 1.** Average± 2SD shapes of *T. versicolor* carpophores visualized with Inverse Fourier Transform, symmetrical coefficients.

PC3 and PC4 (PC5 included in certain cases) correspond to the variation of outline with respect to curvature.

Coefficients of the asymmetrical group describe the asymmetrical variation in curvature containing also the information incapsulated in symmetrical coefficients such as aspect variation PC1 and centroid position (PC2) while PC3 and PV4 describe asymmetrical curvature variations (Iwata et al., 2000).

Summarizing, Fourier descriptors depict the average shape and its variation by including the standard distance of 2SD. To a better understanding of dimensionality and shape of any object reduced to 2D and a closed contour, the statistical analysis is needed (Lootens et al., 2007).

Simple Linear Regression applied on major and minor axis of the carpophores resulted in a linear relationship between the two variables with a slope closer 0 leading to the hypothesis that this relationship is of allometric type, with the reserve of a rather low value for the coefficients of correlation and determination ( $R = 0.7297$  and  $R^2 = 0.5325$ ). The equation of the regression line on log-log transformed data is given by:

$$Y = 0.0164 + 0.6853X + \varepsilon$$

The descriptive statistics for dimensional variables employed in ANOVA and PCA are presented in table 2.

**Table 2**

Descriptive statistics of dimensional variables measured on photographs of *Trametes versicolor* carpophores

variable	mean	SE	SD	Coefficient of variation CV	minimal	maximal
Length (cm)	3.8258	0.0605	1.3664	0.3571	1.2	9.82
Width (cm)	2.6707	0.0390	0.8821	0.3302	0.8	6
Areas (cm <sup>2</sup> )	9.9314	0.7075	7.4715	0.7523	2.57	39.72
Perimeters (cm)	12.5268	0.4979	4.7236	0.3770	6.47	30.66
Aspect (elongation)	1.4587	0.020	0.3352	0.2297	0.90	2.9

A common trend of high variability is observed if analyzing descriptive statistics, with large standard deviations and ranges.

One-way ANOVA applied on dimensional variables revealed extremely significant differences between samples collected from different substrata and locations. Table 3 depicts the F values and significance levels of the comparisons.

**Table 3**

One –way ANOVA results from comparisons performed on samples of *Trametes versicolor* carpophores collected from 3 different substrata (*Prunus sp.*, *Prunus avium*, *Berberis vulgaris*) and 3 locations, taking dimensional variables.

variable	F <sub>calc</sub>	Significance at 0.05 $\alpha$ level	P
areas	29.32 (F <sub>0.05</sub> =3,10)	*** (extremely significant)	P≤0.001
perimeters	31.67 (F <sub>0.05</sub> =3,10)	***	P≤0.001
Short axis	43.91 (F <sub>0.05</sub> =3,10)	***	P≤0.001
Long axis	29.83 (F <sub>0.05</sub> =3,10)	***	P≤0.001
Aspect (elongation)	11.25 (F <sub>0.05</sub> =3,10)	***	P≤0.001

Principal Component Analysis resulted in two most important PC cumulating most of variability as table 4 depicts, conforming to Kaiser criterion, important eigenvalues to be considered have values over 1.

**Tabel 4**

Most important eigenvalues and corresponding variability proportions from Principal Component Analysis of the correlation matrix of areas, perimeters, long axis, short axis and aspects of *Trametes versicolor* carpophores

	PC1	PC2
<b><i>Trametes versicolor</i> - Grigorescu</b>		
Eigenvalues	3.73	0.99
Proportion %	74	19
Cumulative proportion	74	94
<b><i>Trametes versicolor</i> - Hoia</b>		
Eigenvalues	4.06	0.88
Proportion %	81	17
Cumulative proportion	81	98
<b><i>Trametes versicolor</i> - Făget</b>		
Eigenvalues	3.95	1.01
Proportion %	79	20
Cumulative proportion	79	99

For all cases, variables' loadings were on PC1 for area, perimeters, long and short axis and elongation on PC2 meaning that maximum variance is explained for the first 4 variables by PC1 due to high correlation between variables, a reduction by reorientation on new orthogonal axes wasn't possible. However, aspect or elongation is loaded on a different PC as compared to other variables and the explanation is that being a dimensionless variable, it has a different behavior. The important role of this variable is played when considering shape in the frame of Elliptic Fourier Analysis. Pc1 of Elliptic Fourier coefficients corresponds to carpophores' aspect or elongation while the multivariate analysis of dimensional morphometric variables showed that elongation is an independent variable loading on PC2. The PC1 eigenvalues corresponding to asymmetric group of Fourier coefficients are larger than in symmetrical group suggesting that variability in symmetry is rather large.

Observation taken during the study included other xylobiotic fungi associated on the same substratum with *Trametes versicolor*: *Xylaria polymorpha*, *X. carpophila*, *Stereum hirsutum*, *Bjerkandera adusta*, *Calocera virosa*, *Lenzites betulina*, *Trametes gibbosa*, *luteus cervinus*, *Panellus stypticus*, *Kuehneromyces mutabilis*, *Lycoperdon pyriforme*, *Fomes fomentarius*, *Irpex lacteus*, *Phellinus hartigi*. Macromycetes which developed on disinfected and incubated carpophores were: *Cladosporium cladosporioides*, *Arthrobothrys sin ana*, *Basifimbria spinosa*, *Graphium sp.*, *Chaetomium globosum*, *Cladobotryum roseum*. Frequently the carpophores were colonized by algae. *Trebouxia sp.* and *Stichococcus sp.* is considered as proto-symbionts of *T. versicolor* (Zavada et al., 2004). Many samples contained insects inhabiting and feeding on carpophores. We identified *Cis boleti* (Scopoli) and *Octotemnus glabricus* (Gyllenhal). These coleopterans of fam. Ciidae are specialized consumers of wood inhabiting macrofungi, especially *T. versicolor* (Komonen & Kouki, 2005). *Cis boleti* is known to colonize fully developed carpophores while *Octotemnus glabricus* feeds on young carpophores (Guevara et al., 2000).

## DISCUSSIONS

Principal components define a space named as allometric space, a space which is a species characteristic (Lagland et al., 2005). Elongation is a focal variable in the morphometric analysis of shape we performed: it is related to PC1 in Principal Component Analysis applied to Elliptic Fourier Descriptors meaning that it concentrates most of shape variability. It accounts for higher variability in *T. versicolor* as compared to other analyzed biological objects such as *Primula sieboldii* petals, of 48.97% (Yoshioka et al, 2004) or buckwheat kernels of 68.5% (Ninomya et al, 1995) suggesting that it is the most important

shape descriptor in *T. versicolor*. Also, elongation analysis by regressing the two axes of the carpophores shows an allometric relationship between the two variables, which appears during the growth process and is translated by a modification in shape. Multivariate analysis of dimensional variables of the carpophores showed that elongation or aspect varies independently from other dimensional variables since PC1 and PC2 are orthogonal and only elongation loads on PC2. It is an important result since dimensional variables such as area, perimeter, length and width of the carpophores load separately on a different principal component as compared to elongation which is a classical shape descriptor. The trend is common for samples collected from different substrates and locations. Still, dimensional variables and elongation vary significantly between substrates as ANOVA results demonstrate. Different dimensional variables are considered as significant morphometric descriptors which permit the differentiation of geographic or substrate provenances. One such variable is length (Chen & Nelson, 2004) which varies within group (rather high values of SD and CV) and significantly among groups (ANOVA results). Within this variability a common trend in what is defined as morphometric space is observed. Shape analyzed by EFD-PCA is independent of size and detect small outline variations. It is considered that symmetrical shape variations are due more to genetic factors by contrast, asymmetrical variations are determined by environmental factors (Yoshioka et al., 2004). It is a general result of shape analyses using EFA-PCA that PC1 (of Fourier coefficients symmetrical group) corresponds to aspect and PC2 to the position of the centroid (Ninomyia et al., 1995; Hiraoka & Kuramoto, 2004; Yoshioka et al., 2004; Truong et al., 2005; Yoshioka et al., 2007). Asymmetrical shapes described by the eigenvalues of asymmetrical coefficients' group are hypothetically related to fluctuating development asymmetry, a phenomenon encountered often in nature and being determined by environmental stress or hybridization. It consists in small perturbations during developmental processes that take place on right and left sides of the symmetry axis (Klingenberg & Nijhout, 1999) being also an explanation for asymmetries quantified by means of elliptic Fourier descriptors in our study. In the case of *Trametes versicolor* carpophores, the asymmetry is dictated by the development in clusters, the effect of close proximity of other carpophores which modifies the genetic trend toward symmetry. Asymmetrical carpophores are common and the partition of variance among 5 or 6 principal components show that there several independent modes of asymmetry acting at the same time.

High variability between samples collected from different substrata and locations suggest that the form of carpophores (form being the summation of dimension and shape) is influenced at great extent by the environment. Shape *per se* is modeled mainly by aspect and centroid position but the outline is highly variable with respect to lobation or the margin waving described both by higher order PC of the symmetric group and mostly by PC of the asymmetric group.

Dispersal capacity is determined by carpophores shape which creates at their edge, turbulences in the laminar flow of the air, incorporating the falling basidiospores (Moore-Landecker, E., 1972). Fan shaped carpophores encountered in many xilophagous species use edge turbulences to disperse their spores, accordingly it is of great importance how the fertile surface is exposed and how it is disposed on the substrate. The variations in elongation and margin waving permit to expose as much of fertile surface as possible given the clustering effect. A similar principle is encountered in the disposition of leaves on a branch, the subsequent strategy being the maximization of light interception. At the same time, this geometry permits the association with many other organisms looking for shelter or food resource, many invertebrates, microfungi and algal colonizers which opportunistically take advantage of the new space created by carpophores growing in superimposed clusters.



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