Light microscopic studies on pollen grains of selected Cyperaceae species from southern Tamil Nadu, India: Relevance in Holocene sediment studies

S. PRASAD AND K. ANUPAMA

French Institute of Pondicherry, PB 33, 11 St. Louis Street, Puducherry 605 001, India. Email: prasad.s@ifpindia.org

(Received 16 January, 2007; revised version accepted 27 August, 2007)

ABSTRACT

Prasad S & Anupama K 2008. Light microscopic studies on pollen grains of selected Cyperaceae species from southern Tamil Nadu, India: Relevance in Holocene sediment studies. The Palaeobotanist 57(1-2) : 227-233.

Seven species of Cyperaceae have been selected for quantitative pollen morphological studies through light microscopy as a practical application in the reconstruction of Late Holocene vegetation. Such a study has been taken up because this large family has so far been referred to as one pollen type by the palynologists most of whom have attributed wet lands/ marsh lands as the habitat of the members. Though most of the members are found in wet lands, some are found in drier habitats also, making it essential that this family be subclassified, for which a preliminary attempt has been made in this paper in the study area in southern Tamil Nadu, India, that offers a diversity of habitats. The species chosen are *Bulbostylis barbata* (Rottb.) Kunth ex Clarke, *Cyperus compressus* Linn., *C. rotundus* Linn., *Fimbristylis argentea* (Rottb.) Vahl., *F. ovata* (Burm. f.) Kern, *Kyllinga bulbosa* Beauv. and *Scirpus articulatus* Linn. Two subtypes were identified among these seven species, with *Fimbristylis* having distinctive pollen morphological characters.

Key-words—Cyperaceae, Fimbristylis, Pollen subtypes, Tamil Nadu.

भारत, दक्षिणी तमिलनाडु से प्राप्त विशिष्ट साइपरेसी जाति के पराग कणों के प्रकाश सूक्ष्मदर्शिकी अध्ययन ः होलोसीन अवसाद अध्ययनों के साथ प्रासंगिकता

एस. प्रसाद तथा के. अनुपमा

सारांश

अंतिम होलोसीन वनस्पति की पुनर्संरचना में प्रयोगात्मक अनुप्रयोगों के रुप में प्रकाश सूक्ष्मदर्शिकी से मात्रात्मक पराग आकारिकीय अध्ययनों हेतु साइपरेसी की सात जाति चुनी गई हैं। इस प्रकार का अध्ययन इसलिए किया गया है क्यों कि अब तक ये बड़ा कुल परागाणुविदों द्वारा एक पराग की भाँति संदर्भित किया जाता रहा है जिनमें से अधिकांश सदस्यों के आवास के रूप में आर्द्र भूमि/कच्छ भूमि गुणयुक्त हैं। यद्यपि अधिकांश सदस्य आर्द्र भूमि में हैं, कुछ शुष्क आवासों में भी हैं, इसलिए यह जरूरी हो जाता है कि यह परिवार उप वर्गीकृत हो जाना चाहिए, इस ओर इस शोध-पत्र में प्रारंभिक प्रयास किया गया है और अध्ययन हेतु भारत का दक्षिणी तमिलनाडु क्षेत्र चुना गया है जो कि आवासों की विविधता प्रदान करता है। बल्बोस्टायलिश बर्बेटा (रोट्ब) कंठ एक्स क्लार्क, सायपेरस कंग्रेसस लिन., सी. रोटंडस लिन., फिंब्रीस्टायलिश अर्जेटिया (रोट्ब) वहल, एफ. ओवेटा (बर्म.एफ) कर्न क्यलिंगा बलबोसा बेअव और सीर्पस आर्टिकुलेटस लिन. चुनी गईं जाति हैं। इन सात जातियों में से दो उप प्रकार की फिंब्रीस्टायलिस सहित पराग आकारिकीय विशेष गुणों युक्त पाई 1

संकेत-शब्द—साइपरेसी, *फिंब्रीस्टायलिश,* पराग उप प्रकार, तमिलनाडु।

© Birbal Sahni Institute of Palaeobotany, India

INTRODUCTION

POLLEN analysis of a sediment section or core, reflecting the qualitative and quantitative fluctuations in the vegetation assemblages through time, is one of the most powerful tools to reconstruct past environments and ecosystems. Such a reconstruction is always an indirect one the number and type of pollen grains being the concrete observations of this method. Despite proven advantages (Faegri & Iversen, 1989), one of the biggest caveats of pollen analysis remains the level of identification possible. Pollen enumeration and identification are usually done using a light microscope that very often restricts the observation of finer features. It is certainly not practical to identify and count pollen from sediment sections using SEM or TEM. An advantage today is the relatively easy availability of digital photographic attachments with the light microscope that has greatly enhanced the clarity of light micrographs that can be easily exploited for routine identification and counting of pollen in Quaternary sediments.

As it has not always been possible, when dealing with pollen of surface samples or sediments, to identify them at the species level, in this paper, the need for a finer subclassification of pollen types, wherever possible, using morphological characters discernible under the light microscope has been emphasized. Our approach has been to select an ongoing research project that documents for the first time a continuous sediment section ~ 2000 years in the northeast monsoon dominated part of the Indian peninsula, where the need for such a finer distinction was felt for the pollen taxon Cyperaceae. In this study it was found that (along with Poaceae), the pollen of Cyperaceae were quite dominant and the occurrences were showing a definitive pattern, making it a significant marker of monsoon intensity during the Late Holocene (Anupama *et al.*, 2005).

Cyperaceae is one of the largest families in the plant kingdom- third largest family in the monocotyledons after Orchidaceae and Poaceae with ca. 104 genera and 5000 species (Simpson *et al.*, 2003) and their origin dates back to Tertiary Period in tropical and subtropical regions (Raven & Axelrod,

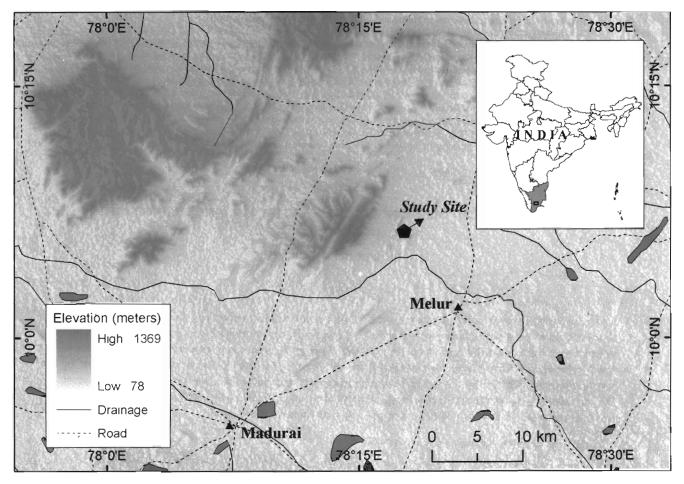


Fig.1-Location of the study site in Tamil Nadu. Inset: location of the study area in India.

	Habitat				
Species	Field observations	Distribution in south India			
Bulbostylis barbata	Mainly in rocky and sandy areas	Open sandy places; all districts; sea level up to 4000'			
Cyperus compressus	In areas adjoining water	Open grassland - moist situations; all districts; sea level up to 6000'			
Cyperus rotundus	Submerged in water and in areas adjoining water	Open lands; all districts; sea level up to 6000'			
Fimbristylis argentea	Submerged in peripheries of water	Wet places, sometimes in clefts of rocks; usually at low elevations, some times up to 4000'			
Fimbristylis ovata	Mainly in open, fallow lands and rocky clefts	Grassland; almost all districts			
Kyllinga bulbosa	In areas adjoining water	All the Eastern and Central districts; up to 3000'			
Scirpus articulatus	Only submerged in water	Tanks and marshy places; chiefly near coast; most districts; up to 3000'			

Fig. 2—Distribution of the studied species in the regional context of south India (Fischer, 1928; Henry *et al.*, 1989) and field observations in the study area.

1974). Cyperaceae has been classified as a single pollen taxon in most of the fossil and modern pollen studies as it has often not been possible to go beyond this with light microscopy. There have been works on subdividing this family into pollen types (Padhye & Makde, 1980; Van Wichelen *et al.*, 1999), but not many attempts have been made to use these studies in relation to sediments.

The link with present day ecology and the past vegetation changes is particularly strong in recent records of the Late Holocene and it is here that a detailed knowledge of the vegetation in and around the sampling site can be applied to a finer distinction of pollen types (Wahl, 2002). Very often pollen identification has been at generic or family levels. Some times when there are no clear characters, in light microscopy, pollen taxa have been assigned names combining two families (e.g.: Moraceae/Urticaceae type, Chenopodiaceae/ Amaranthaceae type). In this context we found that it is possible to distinguish the pollen of Cyperaceae preserved in the sediments into at least two broad categories. It is also possible to assign to these two categories the different species of Cyperaceae shortlisted in this area. The main focus of this paper is to describe their pollen morphology, using light micrographs and to discuss the application of such knowledge in the reconstruction of the Late Holocene vegetation.

STUDY AREA

The study area, located near Melur in southern Tamil Nadu (10°06'N; 78°18'E), India, includes a rain-fed reservoir (tank) and its catchment (Fig. 1). The tank gathers inputs from the surrounding hills and approximates a closed catchment system as the only outlet is a manually regulated sluice for irrigation located on the tank bund. The section (~3 m) representing the sediment run-off harvested within this catchment is described in greater detail elsewhere (Anupama *et al.*, 2005).

METHODOLOGY

Extensive quantitative vegetation studies were carried out in the study area, comprising of the tank, its boundaries and the catchment including adjoining natural forests. Besides, special surveys of the herbaceous taxa were also conducted, from which the species of Cyperaceae occurring in the tank bed and the surrounding areas, in different seasons were identified. Fresh herbarium and pollen collections have been made for each of the species. The floral materials from these specimens were used to prepare pollen slides by acetolysis (Erdtman, 1960) at a temperature of 80°-90°C for 7 to 10 minutes and later mounted with glycerine jelly.

Quantitative studies of pollen grains were carried out using a light microscope by picking out at least 30 wellconserved grains and the observations were made at x1000resolution. All the observed specimens and slides are deposited for further reference at the herbarium of French Institute (HIFP), Puducherry, India.

RESULTS

The vegetation surveys identified seven species of Cyperaceae to be dominant in this region, namely *Bulbostylis barbata* (Rottb.) Kunth ex Clarke, *Cyperus compressus* Linn., *C. rotundus* Linn., *Fimbristylis argentea* (Rottb.) Vahl., *F. ovata* (Burm. f.) Kern, *Kyllinga bulbosa* Beauv. and *Scirpus articulatus* Linn. These seven species have been grouped according to their habitats (Fig. 2) and were selected for quantitative studies on their respective pollen morphology through light microscopy. Their pollen characters have been tabulated (Fig. 3).

Pollen of five of the studied species, *Bulbostylis barbata*, *Cyperus compressus*, *C. rotundus*, *Kyllinga bulbosa* and *Scirpus articulatus* were obovate in outline with a clearly pronounced broad distal end and a tapering narrow proximal

THE PALAEOBOTANIST

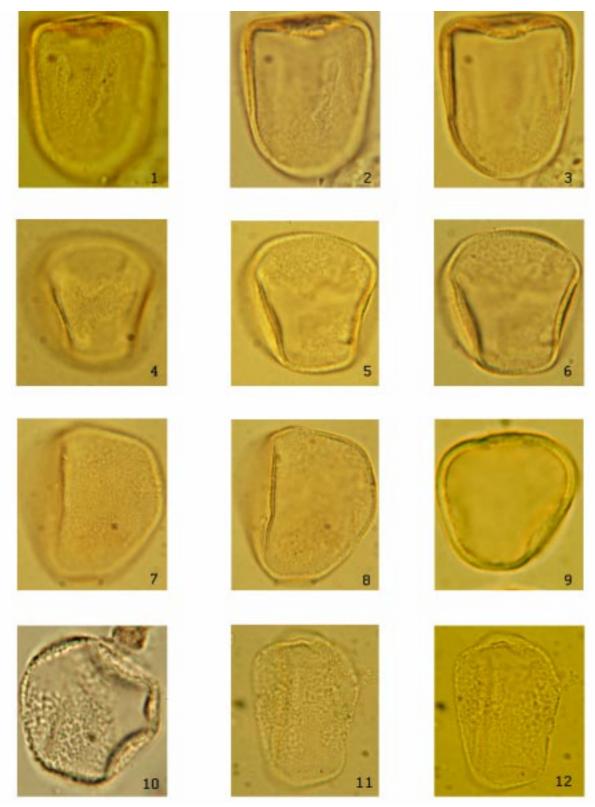


PLATE 1 Light micrographs of selected species of Cyperaceae

1-3.

- Cyperus rotundus Linn. Bulbostylis barbata (Rottb.) Kunth ex Clarke
- 4-6. 7, 8. Scirpus articulatus Linn.

- *Kyllinga bulbosa* Beauv.
 Fimbristylis argentea (Rottb.) Vahl.
 F. ovata (Burm. f.) Kern.

Species	Shape	Length (µm)	Width (µm)	Apertures	Exine Thickn (µm)	Ornamentation ess
Fimbristylis ovata	Spheroidal/oval	29-(36)-50; SD 5.48	22-(27)-37; SD 3.44	Not visible	~1	Scabrate; scabrae arranged in small circles
F. argentea	Prolate-spheroidal /oval	23-(26)-30; SD 2.01	21-(24)-28; SD 2.02	Not visible	~1	Scabrate; scabrae arranged in small circles
Bulbostylis barbata	Obovate	19-(22)-27; SD 2.76	14-(18)-20; SD 2.12	Not visible	0.5-1	Faintly reticulate
Scirpus articulatus	Obovate	36-(40)-42; SD 1.9	31-(34)-36; SD 1.9	Not visible; tendency to form one ulcus at distal end	0.5-1	Faintly reticulate
Cyperus compressus	Obovate	19-(22)-24; SD 1.97	18-(20)-22; SD 1.65	One distal aperture ~6 µm length, 3 to 4 lateral oval diffuse apertures measuring 8.5 x 6 µm	~2	Granulate
C. rotundus	Obovate	23-(34)-40; SD 3.11	22-(26)-31; SD 2.55	One distal aperture ~9 µm length with 3 longitudinal lateral diffuse apertures ~13-15 x 8-10 µm length	1.5-2	Granulate
Kyllinga bulbosa	Obovate	18-(20)-22; SD 1.29	16-(18)-20; SD 1.49	One distal aperture ~6 µm length with 3 lateral oval diffuse apertures measuring 9 x 7 µm	~1	Granulate

Fig. 3-Quantitative pollen characters for seven species of Cyperaceae.

end. The grains were granulate or faintly reticulate with 4-5 apertures (nature of the apertures could not be made out distinctly as they appeared to be depressions), though in two species, *Bulbostylis barbata* and *Scirpus articulatus*, apertures were indistinct.

Pollen of the two species of *Fimbristylis* were apolar and spheroidal to oval in outline with no apertures. The grains were scabrate with thinner exine (Fig. 3).

DISCUSSION

While a detailed investigation of the finer characters requires SEM and TEM studies, most of the analyses (in diverse fields such as Aeropalynology, Paleopalynology, Melissopalynology), consisting of identification and counting, are based on light microscopic observations and hence we seek to explore here the extent to which light microscopic observations can form the basis for distinction of pollen types within Cyperaceae.

During development of pollen grains, with a few exceptions, each pollen mother cell gives rise to four pollen grains initially forming a tetrad. In most plants they are ultimately free from each other: monads. In some genera they do not separate and are dispersed as tetrads or other rarer types of composite grains. In Cyperaceae, three nuclei out of each tetrad degenerate and only one grain develops (Faegri & Iversen, 1989; Kirpes *et al.*, 1996); and the aborted microspores are encased along with the functional microspore in a single pollen wall. The resultant pollen grain is generally known as a pseudomonad. The viable microspore in the pseudomonad is always adjacent to the tapetum, and the three degenerative microspores are located in the pointed adaxial portion of the wedge-shaped pollen grain (Kirpes *et al.*, 1996; Brown & Lemmon, 2000). This unique pattern also explains the shape of the Cyperaceae pollen grains.

The pollen grains are heteropolar with aperturoid areas. They are usually pear shaped or some times spheroidal/ subspheroidal. Many members have similar aperture condition. They are characterized by a single ulceroid aperture occupying practically the entire distal end of the grain. The characteristic type is the pollen where apart from this aperture there may be three or four lateral faintly marked poroid or elongate apertures. They usually possess thin exine that is scantly ornamented with sexine as thick as or slightly thicker than nexine (Padhye & Makde, 1980).

Some workers have distinguished the pollen of this family into at least two types, *Cyperus* type and *Carex* type (Padhye & Makde, 1980). Van Wichelen *et al.* (1999) have worked on the pollen characters of different tribes of the family, suggesting that the group Cyperoideae, to which all the seven species of

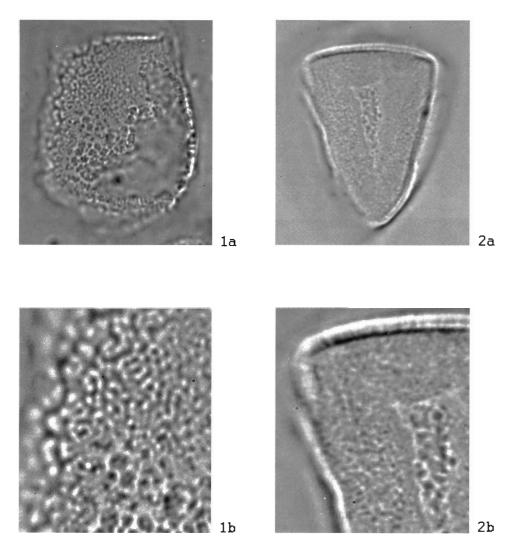


PLATE 2 The two pollen subtypes among the Cyperaceae

1a, lb. Fimbristylis type: The distinctive exine pattern in F. ovata – note the pattern of circles formed by the granulations.

2a, 2b. Cyperaceae type: The "classical" exine of Cyperus rotundus

the present study belong, can be further distinguished based on lateral apertures.

In four of the seven species studied here, *Fimbristylis* ovata, *F. argentea, Bulbostylis barbata* and *Scirpus articulatus*, apertures could not be seen (Pl. 1). Among these four, *B. barbata* and *S. articulatus* have a greater resemblance to the classical shape of Cyperaceae pollen (Erdtman, 1966). Even in the aperturate pollen of this family, the apertures are diffuse and not clear under light microscopes.

From our observations, we have found that the unique pollen type among the species discussed is *Fimbristylis* type, comprising of *F. ovata* and *F. argentea*. The exine in these two inaperturate pollen is scabrate, the scabrae tending to form small circles as revealed under x1000 magnification, whereas in the other species the pattern is not so distinct (Pls 1-2).

Though *Bulbostylis barbata* and *Scirpus articulatus* are also inaperturate and have their unique characters, these may be discernible with observations of reference pollen slides from herbaria, but differentiating them in sediment samples comprising of a heterogeneous mix of pollen types using light microscope will be an uphill task (Pl. 1).

It is suggested that among Cyperaceae, we can call *Fimbristylis* as a unique type and the other Cyperaceae as another type (comprising of *Bulbostylis barbata, Cyperus compressus, C. rotundus, Kyllinga bulbosa* and *Scirpus articulatus*), though more studies are needed to find out if some other species may also come under *Fimbristylis* type.

The members of Cyperaceae are often found in wetlands, swamps and peat bogs (Bush, 2002; Ravi & Mohanan, 2002). The increase of Cyperaceae pollen in fossil samples normally

indicates moister conditions and reflects the development of wetlands such as fens and peat bogs (Kraus et al., 2003). But it will not be prudent to assume that the mere occurrence of this pollen indicates wetlands as some members also occur in upland forests and drier regions (Fischer, 1928; Prasad & Singh, 2002). Few species also occur in mesophytic conditions and in seashore sands. Some species are ephemeral while others thrive all through the year (Bhat & Nagendran, 2001). The interpretation of their occurrences has been confounded by this diversity in habitats. An indication of the diverse habitats of Cyperaceae can be had from the occurrence of the two species of Fimbristylis, with F. ovata occurring in the drier parts of the tank and F. argentea occurring inside water, while Fischer (1928) has noted that this species occurs in clefts of rocks also. The use of this vast family distributed in a diversity of environments as a (pollen) marker of habitat and climate is evidently better served by a finer identification of this family.

The present study does not conclude that only two types are possible to be differentiated in Cyperaceae, as this has covered only the seven species that occur in the study site. More work needs to be done covering different regions and habitats to find out if it is possible to identify further types in this large family.

It is proposed to take up similar works in a few other pollen types, mainly herbaceous taxa like Chenopodiaceae/ Amaranthaceae, Poaceae and Compositae (echinate), to identify further subtypes for a better ecological interpretation of the pollen assemblages.

Acknowledgements—This paper arises out of research supported by a grant from the ISRO-GBP (Indian Space Research Organization's Geosphere Biosphere Programme). At the French Institute of Pondicherry, we thank S Aravajy and L Arul Pragasam for assistance in the field and laboratory; G Jayapalan, N Ravichandran and G Muthusankar for help in finalizing the figures and plates; A Stephen, PV Karunakaran and P Couteron for suggestions on the manuscript. We also thank Anjum Farooqui and an anonymous referee for their critical comments that were valuable in improving the manuscript.

REFERENCES

Anupama K, Thomas JV, Prasad S, Juyal N, Aslam A, Arul Pragasan L & Singhvi AK 2005. Reconstruction of Late Holocene Northeast Monsoon from pollen analysis of sediment sections in a rain-fed irrigation reservoir, Tamil Nadu, India. Poster presentation at PAGES 2nd OSM, Beijing available online: http://www.pages-igbp.org/products/ osmabstracts/AnupamaK.pdf

- Bhat KG & Nagendran CR 2001. Sedges and Grasses (Dakshina Kannada and Udupi Districts). Bishen Singh Mahendrapal Singh, Dehra Dun, India, 341p.
- Brown RC & Lemmon BE 2000. The cytoskeleton and polarization during pollen development in *Carex blanda* (Cyperaceae). American Journal of Botany 87: 1-11.
- Bush MB 2002. On the interpretation of fossil Poaceae pollen in the lowland humid neotropics. Palaeogeography Palaeoclimatology Palaeoecology 177: 5-17.
- Erdtman G 1960. The Acetolysis Method, a revised description. Svensk Bot. Tidskr. 54: 561-564.
- Erdtman G 1966. Pollen Morphology and Plant Taxonomy-Angiosperms. Hafner Publishing Company, New York, 553 p.
- Faegri K & Iversen J 1989. Textbook of Pollen Analysis (4th Edition). John Wiley & Sons, Chichester, 328 p.
- Fischer CEC 1928. Flora of The Presidency of Madras (Vol. III) by J.S. Gamble. Adlard & Son, London, 1347-2017p.
- Henry AN, Chithra V & Balakrishnan NP 1989. Flora of Tamil Nadu, India (Vol. III). Botanical Survey of India, Southern Circle, Coimbatore, 171p.
- Kirpes CC, Clarkst LG & Lersten NR 1996. Systematic Significance of Pollen Arrangement in Microsporangia of Poaceae and Cyperaceae: Review and Observations on Representative Taxa. American Journal of Botany 83: 1609-1622.
- Kraus M, Matthiessen J & Stein R 2003. A Holocene marine pollen record from the northern Yenisei Estuary, southeastern Kara Sea, Siberia. In: Stein R, Fahl K, Fütterer DK & Galimov E (Editors)— Siberian river run-off in the Kara Sea: Characterization, Quantification, Variability and Environmental significance. Proceedings in Marine Science 6: 435-456.
- Padhye MD & Makde KH 1980. Pollen Morphology of Cyperaceae. Journal of Palynology 16: 71-80.
- Prasad VP & Singh NP 2002. Sedges of Karnataka (India) (Family Cyperaceae). Journal of Economic and Taxonomic Botany, Additional Series 21. Scientific Publishers (India), Jodhpur, India, 354 p.
- Raven PH & Axelrod DI 1974. Angiosperm Biogeography and Past Continental Movements. Ann. Mo. Bot. Gard. 61: 539-673.
- Ravi N & Mohanan N 2002. Common Tropical and Sub-Tropical Sedges and Grasses. Oxford & IBH Publishing Co. Pvt. Ltd., New Delhi, India, 219 p.
- Simpson DA, Furness CA, Hodkinson TR, Muthama Muasya A & Chase MW 2003. Phylogenetic relationships in Cyperaceae subfamily Mapanioideae inferred from pollen and plastid DNA sequence data. American Journal of Botany 90: 1071-1086.
- Van Wichelen J, Camelbeke K, Chaerle P, Goetghebeur P & Huysmans S 1999. Comparison of different treatments for LM and SEM studies and systematic value of pollen grains in Cyperaceae. Grana 38: 50-58.
- Wahl E 2002. Paleoecology and testing of paleoclimate Hypotheses in Southern California during the Holocene. Unpublished Ph. D. dissertation, University of Minnesota, 248p.