(GS05) Method in palynology and paleobotany

Date: August 27
Place: Room 5236 (oral) , Room 6325 (poster)

Oral Presentation

Aug. 27 [PM2] Room: 5236

Chairs: Vladimir Torres, Kenneth Neil Mertens

14:30-14:50 **Innovative, non-acid based techniques for palynomorph recovery** GS05-O01 (528)

Vladimir Torres, Niall W. Paterson, John H. Carter, Yow-Yuh Chen, Eric Munoz

14:50-15:10 **Determining the absolute abundance of dinoflagellate cysts in recent marine sediments: further tests of the *Lycopodium* marker-grain method** GS05-O02 (330)

Kenneth Neil Mertens, Andrea Michelle Price,Vera Pospelova

15:10-15:30 **Palynology as a tool in solving tectonic problems: Case study of Tanzanian coastal basin** GS05-O03 (322)

Jean-Frank John Mayagilo

15:30-15:50 **Chemostratigraphic and biostratigraphic analysis of Kiliwani North -1 well S.E. Songosongo island, Tanzania** GS05-O04 (321)

Jean-Frank John Mayagilo

Aug. 27 [PM3] Room: 5236

Chair: Kazuhiko Uemura

16:20-16:40 **The Universal CLAMP Calibration: A First Look** GS05-O05 (492)

Robert Spicer, Jian Yang, Teresa Spicer, Frédéric Jacques, Tao Su, Nan Arens, Elizabeth Kennedy, Alexei Herman, David Steart, Gaurav Srivastava, Rakesh Mehrotra, Paul Valdes, Naresh Mehrotra, Zhekun Zhou, Cheng-Sen Li

16:40-17:00 **Pollen indication of the natural and Chernobyl type geobotanical catastrophes** GS05-O06 (278)

Galina Levkovskaya

Poster Presentation

Aug. 27 [PM1] Room: 6325

13:30-14:30 **Use of DNA-specific stains for studying the fossil plant tissues** GS05-P01 (393)

Igor Ozerov, Nadezhda Zhinkina, Edward Machs, Aleksander Rodionov

New marker grains! - DuPont NEM series is discontinuous now. Here is 'Palynospheres' series from Microsphere Technology Ltd. as a better alternative - GS05-P02 (360)

Takeshi Nakagawa, Ikuko Kitaba, Tom Johnston, *Microsphere Technology Ltd.*, Suigetsu 2006 Project Members

Much faster counting! - PolyCounter 3.1 and Multi Function Panel - GS05-P03 (361)

Takeshi Nakagawa, Suigetsu 2006 Project Members

GS05-O01 (528)

Innovative, non-acid based techniques for palynomorph recovery

Vladimir Torres, Niall W. Paterson, John H. Carter, Yow-Yuh Chen, Eric Munoz

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Since the late 1930's, palynology, the study of pollen, spores and other organic walled microfossils has been used routinely in the oil industry as a tool for biostratigraphic correlation and for the characterization of depositional environments. Among the micropaleontological disciplines, only palynology permits correlation between marine and non-marine strata. The oil industry has traditionally relied upon chemicals such as hydrochloric acid, hydrofluoric acid (HF) and nitric acid to liberate palynomorphs from their host lithologies. We have now developed a new technique for palynomorph extraction that replaces mineral acids such as HF with relatively benign reagents such as sodium hexametaphosphate $(NaPO_3)_6$ and sodium dodecylbenzenesulfonate ($C_{12}H_{25}C_6H_4SO_3Na$), which act as dispersants rather than dissolving the rock matrix. This new processing technique and the results of its application to rocks of a variety of ages and lithofacies will be discussed. Technical contributions of this work include the elimination of HF acid from palynological processing, thereby conferring additional benefits such as reducing overall processing cost and increasing lab performance.

Keywords: pollen, spores, processing techniques, hydrofluoric acid, dispersal agents.

GS05-O02 (330)

Determining the absolute abundance of dinoflagellate cysts in recent marine sediments: further tests of the *Lycopodium* marker-grain method

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Lycopodium clavatum tablets are commonly added as a spike to determine dinoflagellate cyst concentrations in sediments. In this study we investigate the effects of different processing techniques on dinoflagellate cyst concentrations using well-mixed sediment samples from Saanich Inlet, British Columbia, Canada. At the onset of any dinoflagellate cyst investigation, we suggest following the recommendations of Maher (1981) to experimentally adjust the sample size to obtain a ratio close to ~2 of dinoflagellate cysts counted / *Lycopodium* spores counted, in order to obtain reproducible concentrations. Results further show that both oven-drying at ~45 °C and freeze-drying are viable, non-destructive techniques yielding reproducible results. Use of warm HCl (40-60 °C) for a short time (30 minutes) is harmless, whereas treatment with warm HF (40-60 °C) affects the reproducibility of the concentrations. Pre-sieving can result in loss of cysts and/or spike but this can be easily monitored by checking the fraction remaining on the coarse sieve. Perforated metal sieves show more consistent results than the *Nitex* nylon meshes. The use of 30 seconds sonication does not affect the reproducibility and is advised to remove amorphous organic matter. Adding the

Lycopodium spike at the end of preparation yields consistently lower concentrations, which were more than often not reproducible, suggesting noticeable losses of *Lycopodium* spores during processing if the *Lycopodium* spores are added at the beginning. This method can be considered a viable alternative, but the discrepancy should be taken into account.

Keywords: dinoflagellate cyst, concentration, *Lycopodium clavatum* tablets, spike, absolute abundance.

GS05-O03 (322)

Palynology as a tool in solving tectonic problems: Case study of Tanzanian coastal basin

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Palynology has been used as a tool in solving problems in Petroleum exploration, Geology, Archeology and Criminology. Pollen and Spores in Sedimentary strata have been used as indicators of temperature increase with depth. The concept of Spore Colour Index (SCI) has been calibrated to values of Vitrinite Reflectance Index (VRI) and Thermal Alteration Index (TAI) of Sediment maturity in Robertson spore colouration standards(1991). Recent findings have shown that SCI values could be used as tool to indicate presence of Tectonic activity, such as a; fault or unconformity along the traverse of an exploration well or borehole. This was further supported by seismic data or profiles in the vicinity of the wells. Values of SCI in Palynological analyses taken from a number of wells in coastal basins in Tanzania, Mbezi-1 and Minangu-1 suport the concept above. This concept could be applied any where even in sedimentary areas where there no seismic lines or seismic data.

Keywords: spore colour index, vitrinite reflectance, tectonic activity, seismic data, thermal alteration index.

GS05-O04 (321)

Chemostratigraphic and biostratigraphic analysis of Kiliwani North -1 well S.E. Songosongo island, Tanzania

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Chemostratigraphy is the correlation and dating of marine sediments and sedimentary rocks through the use of trace element concentrations,molecular fossils and certain isotopic ratios that can be measured on components of the rocks. Biostratigraphy is a branch of geology dealing with the differentiation of sedimentary rock units on the basis of the fossils they contain. Both methods have been used in analysis of cutting samples from Kiliwani North -1 well located on the South eastern part of Songosongo island in South East coastal Tanzania. Well samples from interval 455m -2015m were analysed using methods mentioned above to determine the stratigraphic boundaries, depositonal environment, sediment provenance,mineral chemical composition and age of samples.

Biostratigraphical results indicate unconformity between Early Miocene and Late Middle Eocene at 1193m and 905m. The Eocene sediments were deposited within proximal neritic/shelf settings, are equivalent to the Pande and Masoko Formations onshore. An Early Eocene age is defined at 1235m, penetrating strata equivalent to the onshore Kivinje Formation deposited within proximal neritic/shelf/marine setting influence. Penetration of the Late Cretaceous, Maastritchian-Late Campanian sediments is indicated at 1652m. Deposition within a general neritic settings is invoked with significant terrigenous input. Some brackish input is also identified. The impoverished assemblage at 1844m precludes determination of stratigraphic relationship between the Late and the Early Cretaceous successions. Samples from 1997m and 2015m yielded Late Barremian palynoflora typical of penetration of the Kipatimu Formation. A proximal depositional setting is inferred. No positive biodata are recorded to indicate the penetration of Pre-Late Barremian strata. Chemostratigraphical results indicate higher quartz content from sample 455m-715m and a down hole decrease from 780m-1095m and an increase at basal section from 1109m to 1298m. Feldspar is least represented (4.5-16%), while calcite is present as cement and not detrital, higher at basal sands from 1109m to 1298m suggesting a calcareous sandstone lithology. Clays are dominant from 780m-1095m indicating marine deposition environment, while heavy mineral content is dominated by hornblende. Sediment provenance changed from dominant Precambrian to Mesozoic source due to both transgressive and regressive episodes. Mineralogical abundance indicates variation in deposition environment and presence of unconformities or hiatuses. Chemostratigraphy presents useful data where biostratigraphy data is unavailable.

Keywords: Chemostratigraphy, Palynoflora, mineralogical abundance, transgressive, regressive and biostratigraphy.

GS05-O05 (492)

The Universal CLAMP Calibration: A First Look

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CLAMP (Climate Leaf Analysis Multivariate Program) is a widely applied and robust palaeoclimate proxy based on the physiognomy of woody dicot leaves. It is not universally applicable, however, because of restrictions arising from the current geographically and climatically limited calibration datasets (http://clamp.ibcas.ac.cn). Because leaf form is demonstrably convergent in similar climates the assumptions underlying CLAMP are that 1) numerous interacting leaf physiognomic traits

influence fitness, 2) natural selection operates towards an optimized 'engineering solution' to maximize photosynthetic efficiency despite often conflicting environmental constraints, and 3) this 'tuning' operates over geologically short timescales (<<1 Ma) by the elimination of ill-adapted taxa, migration and selection for novel genotypes arising by mutation or hybridization. However, these assumptions have not been verified on a global scale. To test whether foliar physiognomic/climate relationships are universal and consistent across the globe we used a diverse dataset (378 sites) collected and scored using CLAMP protocols from vegetation on all continents except Antarctica encompassing a wide spectrum of species, including sites with highly endemic floras, and areas subjected to Quaternary glaciations but minimally impacted by humans. This physiognomic dataset was calibrated against standardized worldwide climate data recorded over a common 30 year interval. Mean Annual Temperature, Coldest Month Mean Temperature, Length of Growing Season, Specific Humidity and Enthalpy show the strongest coding in leaf form. Precipitation variables show more complex relationships arising partly from the gridding process and partly because in wet regimes water conservation only weakly constrains leaf form. The strong correlations between leaf form and temperature-related variables show that plants respond to such variables in a universal manner independent of other climate variables, biogeographic history or phylogeny. This coherence demonstrates that regional variations seen in univariate analyses such as Leaf Margin Analysis disappear when several leaf characters are scored, implying numerous compensatory roles for leaf architectural features. To test the extent to which CLAMP relies on leaf margin characters for temperature estimates we removed all such character states from the global analysis reducing the characters scored to 25. We found that even in the absence of all leaf margin information the CLAMP analysis still revealed a strong correlation ($p << 0.001$) between foliar form and the temperature-related variables, albeit with small increased uncertainty in all cases. Thus it appears that the spectrum of remaining non-margin characters also, collectively, codes for temperature. Overall this global dataset confirms all the assumptions underlying CLAMP outlined above are valid.

Keywords: palaeobotany, palaeoclimate, leaf physiognomy, global correlations.

GS05-O06 (278)

Pollen indication of the natural and Chernobyl type geobotanical catastrophes

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Geobotanical catastrophes are reconstructed on the basis of statistics on abnormal morphology pollen grains. They were accumulated during 53 years of studies. The almost complete disappearance of morphologically typical and normally developed pollen grains is an indicator of stress conditions for the generative spheres of all flower plants of the area, an indicator of a geobotanical catastrophe. According to the SEM micrographs the complexes of natural geobotanical catastrophes look like the "cemeteries" of hundreds of pollen contours with two pathologies in each form (underdeveloped and dwarf) and the Chernobyl complexes like the "cemeteries" of forms with tree pathologies (defective, underdeveloped and dwarf/giant). Such complexes some palynologists interpret as "re-deposited", but as a result of re-deposition the dwarf, asymmetrical or other abnormal forms could not appear. The ecology of natural complexes with domination of abnormal pollen grains was studied on the basis of multidisciplinary data. They were discovered for Pleistocene glacials and stadials from different regions (Altai, Transcarpathia, Caucasus, Russian plain). But these complexes are rare in interglacials, including Holocene, because the flower plants occupy major territories and their pollen is transported at long distances. Data on subfossil pollen

spectra from different geobotanical sub-zones of West Siberia [Levkovskaya, 1973] and from South Arabia [Levkovskaya, Filatenko, 1992] show that high domination of underdeveloped pollen grains is registered only in rare samples from Southern parts of desert and tundra zones. It is important to collect statistics about individual specificity of each pollen grain in all studied samples and to prepare additional graphs with percentages of the sums of all morphologically normal and abnormal grains of different taxa and percentage of: 1. underdeveloped, 2. dwarf, 3. defective forms and 4. pollen with combination of all these pathologies.

Keywords: ecological crises, defective palynomorphs statistics, underdeveloped (abortive) pollen statistics.

GS05-P01 (393)
Use of DNA-specific stains for studying the fossil plant tissues

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The investigation was based on the Early Eocene myrtaceous leaf and fruit remains collected in the north of East Siberia as well as dicotyledonous remains from the digestive tract of a Late Pleistocene mammoth discovered in the same area. The aim of the study was to detect the DNA-containing structures in the tissues of the fossil plants, possible thanks to the occasional preservation of cells containing nuclear structures in some compressions. The permanent preparations of the fossilized plant tissues were made according to the common cytoembryological procedure. A triple staining was applied to the permanent preparations: Feulgen reaction, the Ehrlich's haematoxylin and the alcian blue, the latter two used as stains for the cell walls and the cytoplasm.The Feulgen-positive unevenly stained nucleic structures have been shown to preserve in the fossilized plant tissues. The chromocenters are stained more intensively. The nuclei have red color that attests the preservation of deoxyribose in the nuclear chromatin. Some cells within an unstained zone surrounding the nucleole have well discernible Feulgen-positive rounded bodies that possibly originated due to the postmortal aggregation of the chromatin. In addition, staining with the Ehrlich's haematoxylin and the alcian blue reveals all the cell structural characters: cell walls (bright blue), callose in the cell walls (light to dark blue), cytoplasm (grayish lilac of varying intensity) and nuclear envelopes (dark violet). The results show that the nuclear material preserved in the fossils is morphologically similar to the chromocenters in the modern plants and has a Feulgen-positive red coloring that testifies the presence of deoxyribose. A relatively bright fluorescence of rounded structures morphologically similar to nuclei is observed after staining with the nucleotide-specific fluorochromes Hoechst 33258 and DAPI. This work was partially financed by the Russian Foundation for Basic Research (grant # 11-04-01953).

Keywords: Early Eocene, Late Pleistocene, plant compression fossils, ancient DNA, Feulgen reaction.

GS05-P02 (360)
New marker grains! - DuPont NEM series is discontinuous now. Here is 'Palynospheres' series from Microsphere Technology Ltd. as a better alternative -

Takeshi Nakagawa[1], Ikuko Kitaba[2], Tom Johnston[3], *Microsphere Technology Ltd.*[3], Suigetsu 2006

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Marker grain method is commonly used by palynologists of the world to quantify absolute pollen concentration in sediments. *Lycopodium* tablets and DuPont NEM microbeads are two of the most commonly used marker grains. DuPont NEM series has some obvious advantages over *Lycopodium* tablets such as very high visibility (it is matt black and spherical), perfectly exotic nature in the ecosystem (*Lycopodium* spore, on the contrary, may naturally exist in sediments), and availability in different size ranges. However, regretfully the product was discontinued several years ago and the users of the DuPont NEM series are now 'hunting' dead stocks of the product on the shelves of the international resellers. Here we propose a new alternative range of marker grains. The 'Palynospheres' series of microbeads, developed specially for palynologists by Microsphere Technology Ltd., Ireland, is tolerant to HF as well as all other chemical and physical stresses during pollen slide preparation. The particles are matt black, spherical, available in well considered size ranges and have a density very close to the fossil pollen grains (*c.* 1.4 g/cm^3). The product is available either as dry powder in different size ranges, or as a mixture of two size ranges blended in a fixed ratio and dispersed in heavy liquid in precisely controlled quantities. The latter product, termed 'Suigetsu special blend', is a standard marker grain solution developed for the Suigetsu 2006 Varved Sediment Core Project (www.suigetsu.org), and has been tested on hundreds of sediment samples analysed during that project. It is prepared in such a way that it is ready to be added to sediment samples without further manipulation. Mixing two different size ranges in a known ratio serves to detect any laboratory failures that differentially favour recovery of bigger or smaller pollen types. For further details or sales inquiries, please email <info@microspheretechnology.com> or visit the Company's web site at <www.microspheretechnology.com>.

Keywords: marker grain, microbeads, Palynospheres, absolute pollen concentration.

GS05-P03 (361)

Much faster counting! - PolyCounter 3.1 and Multi Function Panel -

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PolyCounter software is a powerful tool to assist fast microfossil analyses. Since its reveal at the 2008 IPC in Bonn, PolyCounter has slowly but steadily gained users and now boasts more than 300 downloads in more than 50 countries. The aim of this poster is to provide updates about the software for the last inter-congress period. Firstly, PolyCounter has been updated from ver. 1.5 in Bonn to ver. 3.1 (as of March 2012). In addition to the basic functions of counting up to 135 taxa by a single action, drawing a pollen diagram, subtotal alarm, and output in Excel spreadsheet format both in raw counts and percentages, the updated software now allows (i) plotting a diversity saturation diagram, (ii) keeping 6 additional parameters in digits or texts, (iii) CONISS analysis to assist pollen zoning, and (iv) changing the sample order on the pollen diagram using mouse. PolyCounter 3.1, like

previous versions, is a freeware and is not protected by copyright. Secondly, the input device recommended in Bonn (Ergodex DX-1) does not support Windows Vista or 7. The Solution to this problem is to use the Multi Function Panel (MFP) distributed by CH Products. MFP uses Ergodex technology and supports both Windows Vista and 7. MFP is slightly more expensive than DX-1, but still retails for less than $300 including additional keys No. 26-50 and shipping costs (*i.e.* still cheaper than a number of mechanical tally counters). Demonstration of the updated system will be available at the poster. PolyCounter 3.1 can be downloaded from <http://dendro.naruto-u.ac.jp/~nakagawa/>. Multi Function Panel can be purchased from: <http://www.chproducts.com/shop/usb.html>.

Keywords: PolyCounter, Multi Function Panel, Ergodex DX-1.