Morphological Identification and Diversity Analysis of Fossil Diatoms from Diatomite Sangiran Central Java Indonesia
(Identifikasi Morfologi dan Analisis Keanekaragaman Fosil Diatom dari *Diatomite* Sangiran Jawa Tengah Indonesia)

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ABSTRACT
Diatomite Sangiran is diatom fossil rich sediment. The aim of this research was to study the diversity of fossil diatoms from Sangiran, Indonesia based on morphological characteristics. Samples were taken from lower, middle, and upper sediment layers based on their different physical features with three replications each. Diatomite extraction was performed following modification of Setty (1966) and frustule counting was accomplished by census method. There were total 50 species found in diatomite layers. The diversity analysis showed that highest species richness (21-22), diversity (1.35-1.47), and evenness index (0.44-0.48) were belong to the lower layer. The highest frustule abundance ($9.66x10^7$-$1.43x10^8$ frustules/gram) and dominance index (0.67-0.72) were belong to the middle layer. On the other hand, highest centrales:pennales ratios (0.73-1.11) were belong to the upper layer. The sediment layers signified an obvious indication of gradual changes from marine to freshwater environment. Dendrogram analysis using MINITAB.v.15.1.2 software denoted similarity between lower sediment layer and the younger layers was 72.12%; while similarity between middle and upper layers 92.63%.

Keywords: diatom, diatomite, diversity, morphology, Sangiran

ABSTRAK
Diatomite Sangiran merupakan sedimen yang kaya fosil diatom. Tujuan penelitian ini adalah menganalisis keanekaragaman fosil diatom dari Sangiran, Indonesia berdasarkan karakteriktik morfologis. Sampel diambil dari lapisan bawah, tengah, dan atas berdasarkan ciri fisik yang berbeda dengan masing-masing tiga ulangan. Ekstraksi *diatomite* dilakukan mengikuti modifikasi metode Setty (1966) dan penghitungan frustul dilakukan dengan metode sensus. Total 50 spesies ditemukan dari ketiga lapisan *diatomite*. Analisis keanekaragaman menunjukkan kekayaan spesies (21-22), indeks keanekaragaman (1.35-1.47), dan keseragaman (0.44-0.48) tertinggi dimiliki oleh lapisan bawah. Kelimpahan frustul ($9.66x10^7$-$1.43x10^8$ frustul/gram) dan indeks dominansi (0.67-0.72) tertinggi dimiliki oleh lapisan tengah. Rasio centrales:pennales (0.73-1.11) tertinggi dimiliki oleh lapisan atas. Lapisan-lapisan sedimen tersebut mengindikasikan perubahan lingkungan secara berkala dari laut menjadi tawar. Analisis dendrogram menggunakan *software* MINITAB.v.15.12 menunjukkan kemiripan lapisan bawah dengan dua lapisan yang lebih muda adalah 72.12%, sedangkan lapisan tengah dan atas mempunyai kemiripan sebesar 92.63%.

Kata Kunci: diatom, *diatomite*, keanekaragaman, morfologi, Sangiran

INTRODUCTION

Diatom is a group of unicellular golden brown algae inhabiting light-exposed zones of entire aqueous and semi-aqueous environments. Diatom consist of two orders, Pennales (bilateral symmetry) and Centrales (radial symmetry). External box-like skeleton (frustule) of diatom comprise of two overlapping silica valves. Classification of diatoms is based on frustule shape and patterns-types of surface ornamentation such as pores, areolae, spines, ridges, etc. (Barron 1987). Dead diatom frustules form sediments at the base of their habitats (lake, river, sea, ocean, etc). These sediments still existed million years later and become diatomite through fossilization.

Color indicates purity of diatomite. White represents high purity of diatomite. Diatomite color is commonly off-white to gray, it is rarely black. Diatomite can be further described as chalk-like, soft, friable, and very finely porous or very low in density (floating on water until saturated) (Moyle & Dolley 2003). According to Soeprobowati *et al.* (2010), diatoms can be fossilized as diatomite because their silica contents are more than 90%. Diatomite in Indonesia can be found at majority of fossil-rich sites, such as Sangiran.

Fossil-rich site of Sangiran, known as Sangiran Dome, is located in Central Java Province, 12-20 km north of Solo City. A series of mud volcano eruptions made the Dome's center breached out. The Dome is subdivided into four formations: Kalibeng, Pucangan, Kabuh, and Notopuro (Itihara *et al.* 1985). Bettis *et al.* (2009) stated that nowadays those formations are known as Puren, Sangiran, Bapang, and Pohjajar. Research by Itihara *et al.* (1985) showed that diatoms can be found at Sangiran (Pucangan) Formation. Bettis *et al.* (2009) and Bettis *et al.* (2004) studied the age of Sangiran Formation using $^{40}Ar/^{39}Ar$ analysis resulting that the age is between 1.66 and 1.90 ± 0.02 million years.

Knowledge about diatom deposition in sediment has many applications and uses, such as for indication of petroleum deposit (Smol and Stoermer 2010). Diatoms preserved in marine sediments are commonly used to reconstruct paleoenvironments and paleoceanographic events for Holocene and Quaternary (Jordan & Stickley 2010). Van Den Hoek *et al.* (1995) stated that diatom fossil assemblages can be used to determine whether the sediments are of marine or non-marine origin. To achieve this, of course, it must be assumed that species had the same ecological preferences in the past that can be observed today.

Many research in Sangiran focused on molluscs, foraminiferans, vertebrates, and hominids fossils. However, focused research on morphological identification and diversity analysis of diatom fossil are rare and the publications are difficult to be found.

The objective of this research was to study diversity of diatoms fossil from Sangiran, Sragen, Central Java, Indonesia based on the morphological characteristics and to estimate sedimentation process of the diatomite.

MATERIALS AND METHODS

This research was conducted on January-July 2013. Diatomite samples were taken from Pablengan -Krikilan Village, Sangiran Dome, Sragen Regency, Central Java, Indonesia. Preparation of samples and slides was conducted in Microtechnique Laboratory of Biology Department, Faculty of Mathematics and Natural Science, Bogor Agricultural University, Indonesia. Sample identification and counting was conducted in Biomikro 1 Laboratory of Department of Aquatic Resources Management, Faculty of Fisheries and Marine Science, Bogor Agricultural University, Indonesia.

Samples were taken from lower, middle, and upper diatomite layers from Sangiran Formation (Figure 1). The layers were differentiated based on their physical features. The lower layer is a grey-dark diatomite with high hardness because it were mixed with other sediment of Sangiran Formation. The middle layer is a white and less hard diatomite, showing that this layer has high purity of diatom. The upper layer is a white-brownish and less hard diatomite mixed with alluvial sediment.

Samples taken from the three layers were prepared following modification of Setty (1966). Those samples were dried overnight at 125ºC in an oven and then cooled to room temperature. After drying and cooling, sample from lower layer were seperated because of its high hardness. Samples from upper and middle layers were taken out 200 mg each and added with 33 mL 7.5% hydrogen peroxide (H_2O_2) using beaker glass 250 mL. This procedure was conducted in afume hood. Samples were boiled for 10 minutes in a hot waterbath. After samples were cooled, 200 mL distilled water were added to the samples and allowed to stand for 24 hours. Supernatant were carefully discarded prior to addition of 33 mL 12.5% hydrochloric acid (HCl). The suspension were boiled back for 7.5 minutes. After boiling and cooling, 200 mL distilled water were added before letting back to stand for 24 hours. Supernatant were carefully discarded and 200 mL distilled water were added for 24 hours. Following that, 33 mL 12.5% nitric acid (HNO_3) and 200 mL distilled water were added and stood back for 24 hours. Before the supernatant were discarded and the residual samples were transferred carefully into 1.5 L beaker glass. Finally, 1.1 L distilled water were added to the residue and let to stand for 24 hours. This last step was repeated 5 times.

The extraction procedure for the lower layer was different, particularly in terms of the acid liquid for lower layer sample were hydrogen peroxide (H_2O_2) 15% for 20 minutes, hydrochloric acid (HCl) 25% for 15 minutes, and nitric acid (HNO_3) 25%. The remaining steps were the same as the upper and the middle layer above.

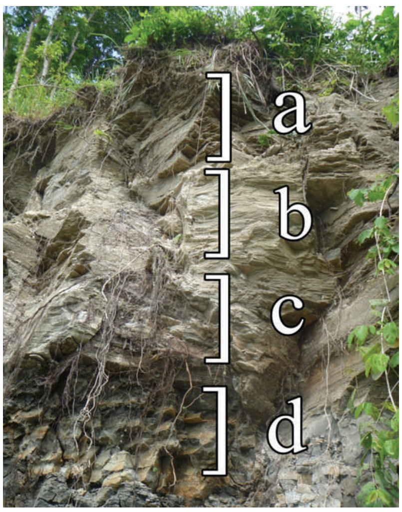

Figure 1. Diatomite Layers of Sangiran Structure of diatomite sediment layers: (a) upper diatomite layer, (b) middle diatomite layer, (c) lower diatomite layer, (d) other part of Sangiran Formation.

Slides were prepared following modification of Setty (1966). The residue of the prepared sample was mixed with distilled water 40 mL. Then, 0.05 mL suspension were deposited on cover glass and dried on hot plate. This volume represented 0.025 mg of the sediments. Then, a very small drop of entellan mounting medium was added on each corner of cover glass and patched carefully on labeled object glass. Three cover glasses from each sample were prepared as replications.

Diatom identification was completed using Prescott (1954), Yamaji (1966), Desikachary (1986, 1988, 1989). Counting was carried out using census method. Frustules in fair condition (seen at least ≥ 50% of the original shape) and poor condition were counted differently to reduce error counting (fair frustule was counted as 1 and poor frustule as 1/2). Then, the obtained data of two counting processes were merged into one data. Because each replication represented 0.025 mg of the sediment, number of frustule per gram were acquired by formula:

Number of frustule per gram = counting result x 40000

Frustule observation was completed using Primo Star iLED microscope and AxioCam ERc 5s microscope camera at 400x magnification power. Images of the observed diatoms were captured using Axio Vision Rel. 5.8 software.

Data from identification and counting result was analyzed according to number of species, abundance, diversity, and evenness index using Shannon-Wiener diversity index, and dominance index using Simpson dominance index (Dash 2001). Ratios of number of centrales:pennales (c:p) were analyzed following Cooper (1995).

According to Wilhm and Dorris (1968), values of diversity index using ln function are divided into 3 categories, i.e low (H'< 2.3026), moderate (2.3026≤ H'≤ 6.9078), and high (H'>6.9078)

The value of evenness index lies between 0 (minimum evenness) and 1 (maximum evenness) (Dash 2001).

Near zero value is obtained when numerous species are present and maximum value 1 is in the case of complete dominance (Dash 2001).

Dendrogram based on similarity among each sediment layer was constructed using MINITAB. v.15.1.2. Used variable of similarities were species and its average frustule density.

RESULTS

Lower Diatomite Layer

Lower diatomite layer contained the largest number of species among the layers. Total 34 number of species, ranging from 21-22 species per replicate, were found from lower diatomite layer (Table 1). Figure 2 shows that lower diatomite layer has *Coscinodiscus kutzingi* as the most abundant species with average frustule amounted to 2.42x10^7 frustules/gram, and followed by *Fragilaria* sp1. as the second with average frustule amounted to 1.68x10^7 frustules/gram. The layer had diatom abundance, counted by total frustule per replicate, ranging from 5.30x10^7-5.88x10^7 frustule/gram. The diversity index ranged from 1.35-1.47; while the evenness index ranged from 0.44-0.48. Dominance index of the layer ranged from 0.29-0.32. Ratios of c:p ranged from 0.38-0.40.

Middle Diatomite Layer

There were 27 number of species with 16-22 species per replicate found from middle diatomite layer (Table 1). Figure 3 shows that middle diatomite layer had *Fragilaria* sp1. as the most abundant species with average frustule amounted to 1.03x10^8 frustules/gram. The second abundant species, *Coscinodiscus lineatus,* has much lower average frustule number compared with *Fragilaria* sp1. i.e 1.03x10^7 frustules/gram. The layer had diatom abundance, counted by total frustule per replicate, ranging from 9.66x10^7-1.43x10^8 frustule/gram. The diversity index ranged from

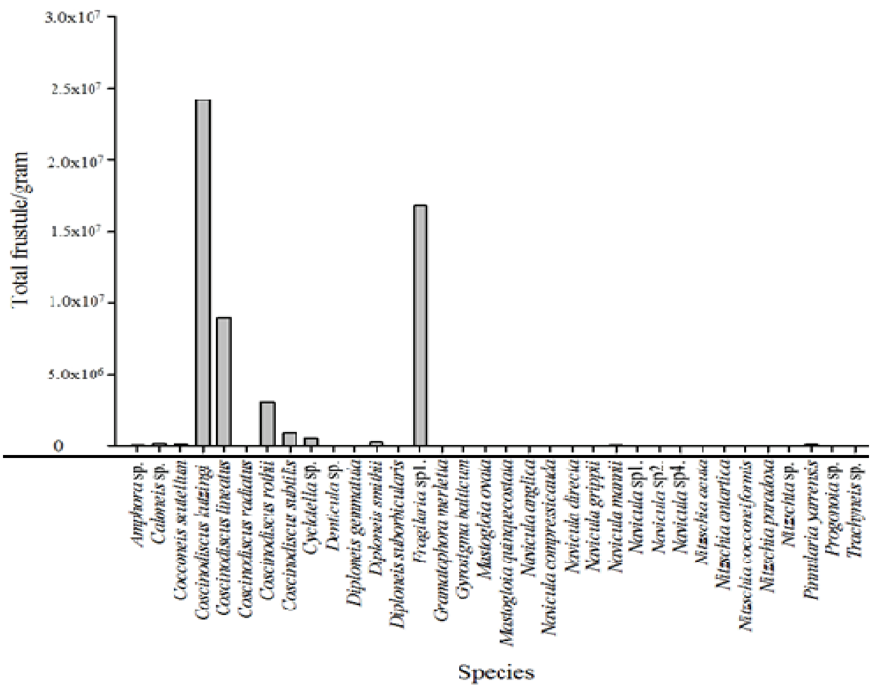

Figure 2. Diatom species and frustule amount found at lower diatomite layer of Sangiran Formation

Table 1. List of diatom species from lower, middle, and upper diatomite layers.

Species	Lower	Middle	Upper	Species	Lower	Middle	Upper
Amphora sp.	+	-	-	*Mastogloia ovata*	+	-	-
Caloneis sp.	+	+	-	*Mastogloia quinquecostata*	+	-	-
Cocconeis scutellum	+	-	+	*Navicula anglica*	+	-	+
Coscinodiscus biangulatus	-	+	+	*Navicula compressicauda*	+	-	-
Coscinodiscus bulliens	-	-	+	*Navicula directa*	+	-	-
Coscinodiscus curvatulus	-	+	+	*Navicula grippii*	+	-	-
Coscinodiscus kutzingi	+	-	-	*Navicula mannii*	+	+	-
Coscinodiscus lineatus	+	+	+	*Navicula* sp1.	+	+	-
Coscinodiscus radiatus	+	+	+	*Navicula* sp2.	+	-	-
Coscinodiscus rothii	+	+	+	*Navicula* sp3.	-	-	+
Coscinodiscus subtilis	+	+	+	*Navicula* sp4.	+	+	-
Cyclotella sp.	+	+	+	*Nitzschia acuta*	+	+	+
Cymatodiscus planetophorus	-	+	+	*Nitzschia antartica*	+	+	+
Denticula sp.	+	+	-	*Nitzschia cocconeiformis*	+	-	-
Diploneis crabro	-	-	+	*Nitzschia granulata*	-	+	-
Diploneis gemmatula	+	+	-	*Nitzschia paradoxa*	+	-	+
Diploneis gruendlerii	-	+	-	*Nitzschia* sp.	+	-	-
Diploneis smithii	+	+	+	*Pinnularia yarrensis*	+	+	+
Diploneis suborbicularis	+	+	+	*Progonoia* sp.	+	-	-
Fragilaria sp1.	+	+	+	*Pseudoeunotia doliolus*	-	-	+
Fragilaria sp2.	-	+	+	*Rhopalodia* sp.	-	-	+
Gephyra media	-	+	-	*Schuettia annulata*	-	-	+
Gramatophora merletta	+	-	+	*Stictodiscus argus*	-	+	-
Gyrosigma balticum	+	-	-	*Surirella* sp.	-	+	+
Lycmophora sp.	-	+	-	*Trachyneis* sp.	+	-	-

0.68-0.77; while the evenness index ranged from 0.22-0.27. Dominance index of the layer ranged from 0.67-0.72. Ratios of c:p from this layer ranged from 0.57-0.80.

Upper Diatomite Layer

There were 26 number of species with 14-19 species per replicate found from upper diatomite layer (Table 1). Figure 4 shows that upper diatomite layer has *Fragilaria* sp1. as the most abundant species with average frustule amounted to 2.37×10^7 frustules/gram followed by *Cyclotella* sp. as the second with average frustule amounted to 1.55×10^7 frustules/gram. The layer had diatom abundance, counted by total frustule per replicate, ranging from 4.49×10^7-1.19×10^8 frustule/gram. The diversity index ranged from 1.03-1.11; while the evenness index ranged from 0.35-0.42. Dominance index of this layer ranged from 0.41-0.45 and ratios of c:p ranged from 0.73-1.11.

DISCUSSION

Dendrogram of cluster variables of three diatomite layers (upper, middle, and lower) was constructed to show similarity among the layers. Figure 5 shows that upper and middle diatomite layers were closely similar in 92.63%. On the other hand, lower diatomite layer was different from two other layers with similarity 72.12%. It indicates that the lower diatomite layer was different from the younger layers.

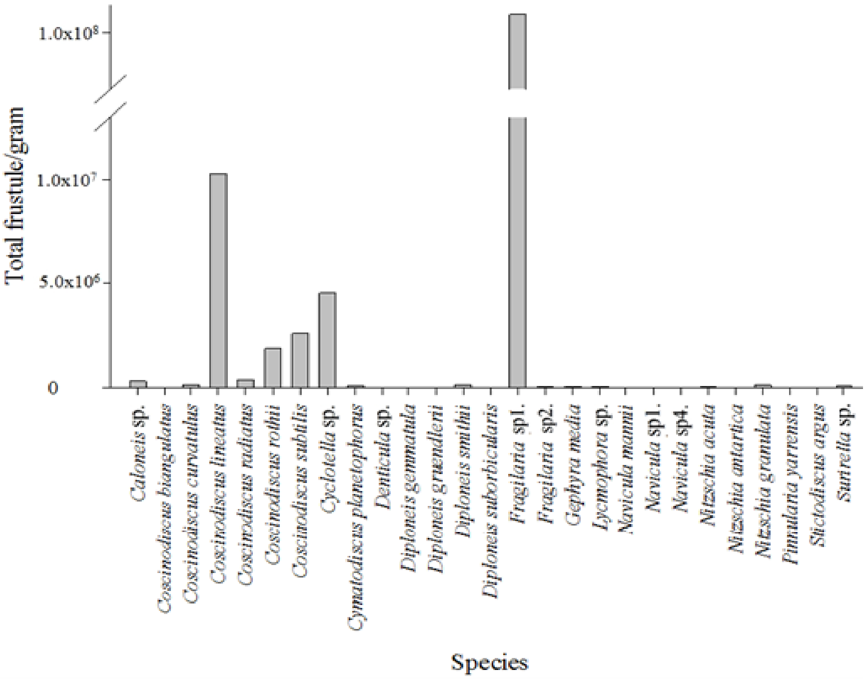

Figure 3. Species which has the most abundant frustules from middle diatomite layer: A) *Fragilaria* sp1., B) *Coscinodiscus lineatus.* Scale bars = 10 μm.

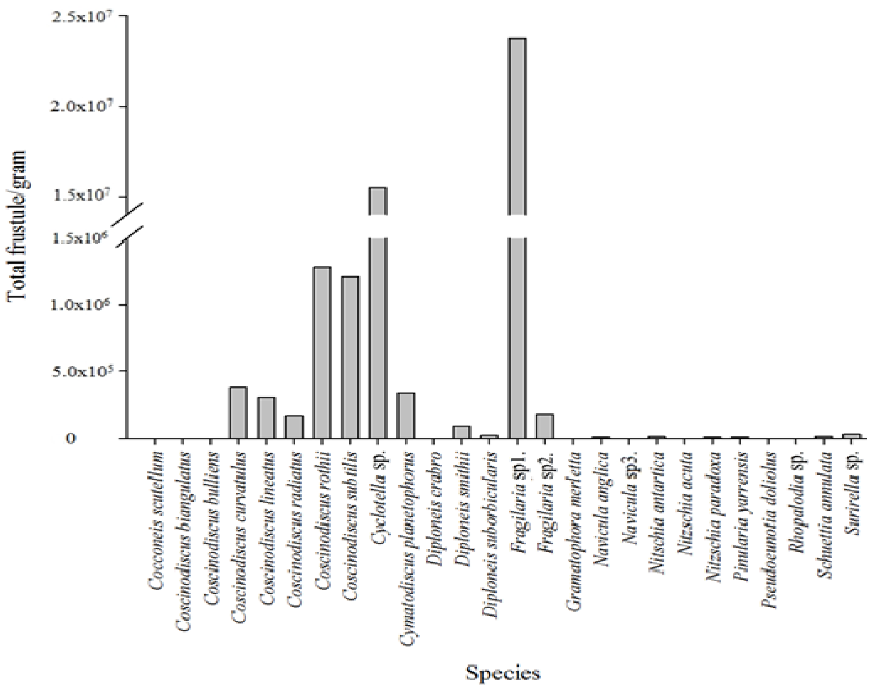

Figure 4. Diatom species and frustule amount found at upper diatomite layer of Sangiran Formation.

Sedimentation process always follows superposition law. Upper layer is naturally formed last, consequently it is the youngest sediment compared to other layers (Soeprobowati 2010). Given to this condition, lower diatomite layer is the oldest. The layers could show different characters because of the differences in forming age.

Diatom fossil assemblages can be used to determine marine or non marine origin of the sediments (Van Den Hoek *et al.* 1995). Lower diatomite layer

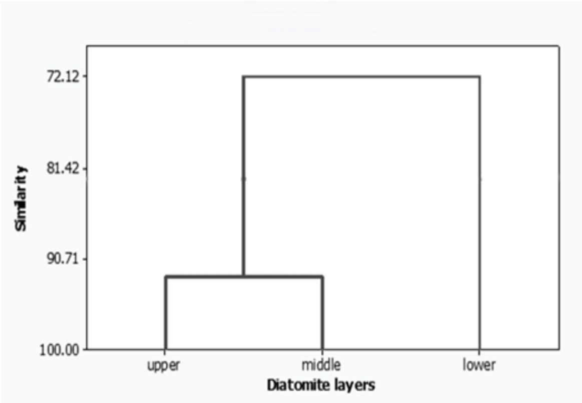

Figure 5. Dendrogram of cluster variables of three diatomite layers (upper, middle, and lower)

contained *Coscinodiscus kutzingi* as the most abundant species and closely followed by *Fragilaria* sp1. According to Prescott (1954), most of *Coscinodiscus* species belong to marine. Hasle *et al.* (1996) stated that most *Fragilaria* species belong to freshwater. It means that lower diatomite layer origin could be presumed as a mix environment of marine and freshwater. Middle diatomite layer contained *Fragilaria* sp1. as the most abundant species and followed distantly by *Coscinodiscus lineatus.* Therefore, middle diatomite layer origin could be presumed as freshwater slightly mixed with marine. Upper diatomite layer contained *Fragilaria* sp1. as the most abundant species and *Cyclotella* sp. as the second. Hasle *et al.* (1996) also stated that most *Cyclotella* species belong to freshwater. Therefore, upper diatomite layer origin could be presumed as freshwater.

The existence of diatom can be described by several approaches, such as diversity and richness. Diversity indices usually consist of diversity, evenness, and dominance index (Dash 2001 and Magurran 1988). This study revealed that order of layers from least to highest in diversity and eveness index were middle (0.68-0.77; 0.22-0.27), upper (1.03-1.11;0.35-0.42), and lower layer (1.35-1.47;0.44-0.48); which were considered to be low values. The order in dominance index was lower (0.29-0.32), upper (0.41-0.45), and middle layer (0.67-0.72). Lower and upper layers were considered to have low dominance, while middle layer has high dominance. Frustule abundance of lower, upper, and middle layers were $5.30x10^7$-$5.88x10^7$, $4.49x10^7$ -$1.19x10^8$, and $9.66x10^7$-$1.43x10^8$, respectively. In species richness, the lowest was found in the upper layer (26 species), followed by middle layer (27 species), then lower layer (34 species). There were total 50 species found from the three diatomite layers. The light microscopic illustration of all diatom taxa is in Figure 6, 7, and 8.

Ratios of centrales:pennales can indicate planktonic or benthic habitats. Lower ratio indicates a tendency to benthic habitat; in contrast, higher ratio tends to be more planktonic habitat (Cooper 1995). Lower diatomite layer has the lowest c:p ratios (0.38-0.40), middle layer has ratios 0.57-0.80, and upper layer has the highest ratios (0.73-1.11). Therefore, order of layers based on habitats was lower layer (more benthic), middle layer (more benthic-planktonic), and upper diatomite layer (more planktonic).

Each part of the diatomite layers from Sangiran Central Java showed different characters. This is an indication of environmental change. First, lower layer is originated from more benthic marine mixed with freshwater diatoms which has the highest indices of diversity, evenness, and species richness while it has lowest dominance index and frustule abundance. Then, middle layer was characterised by more benthic-planktonic freshwater slightly mixed with marine diatoms which has highest dominance index and frustule abundance. Meanwhile, the middle layer has a fair species richness but lowest in diversity and evenness index. Finally, upper layer contains more planktonic freshwater diatoms with lowest species richness but fair in diversity, evenness, dominance, and frustule abundance. However, the dendrogram show a high similarity between the upper and middle layer comparing to the lower layer.

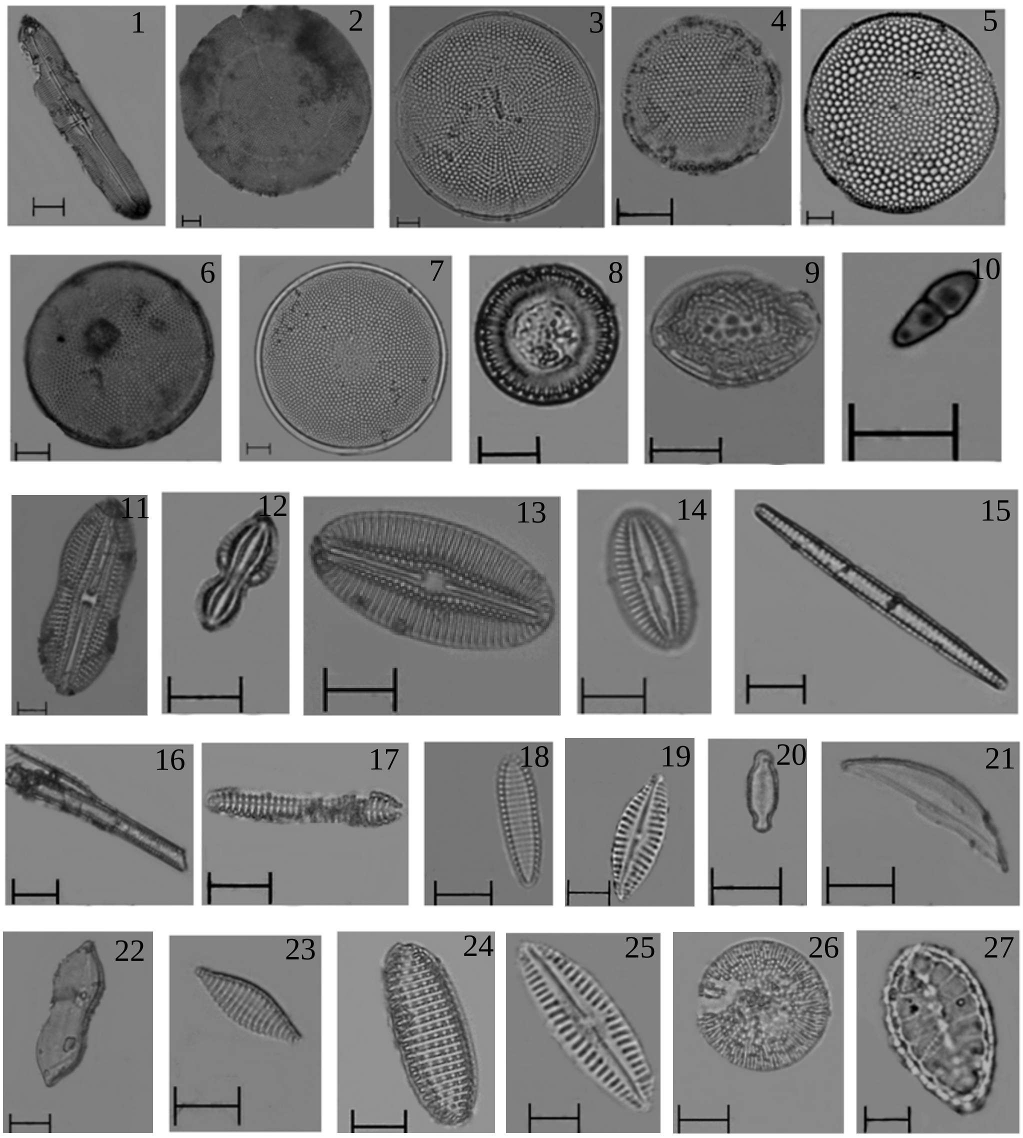

Figure 6. Alphabetical list of diatom taxa from middle diatomite layer

Remarks: 1. *Caloneis* sp.; 2. *Coscinodiscus biangulatus;* 3. *Coscinodiscus curvatulus;*4. *Coscinodiscus lineatus;* 5. *Coscinodiscus radiatus;* 6. *Coscinodiscus rothii;* 7. *Coscinodiscus subtilis;* 8. *Cyclotella* sp.*;* 9. *Cymatodiscus planetophorus;* 10. *Denticula* sp.; 11. *Diploneis gemmatula;* 12. *Diploneis gruendlerii;* 13. *Diploneis smithii;* 14. *Diploneis suborbicularis;* 15. *Fragilaria* sp1.; 16. *Fragilaria* sp2.*;* 17. *Gephyra media;* 18. *Lycmophora* sp.; 19. *Navicula mannii;* 20. *Navicula* sp1.; 21. *Navicula* sp4.; 22. *Nitzschia acuta;* 23. *Nitzschia antartica;* 24. *Nitzschia granulata;* 25. *Pinularia yarrensis;* 26. *Stictodiscus argus;* 27. *Surirella* sp. Scale bars = 10 μm

show a high similarity between the upper and middle layer comparing to the lower layer.

Result shows an obvious indication of environment change of sediments from marine to freshwater. However, Itihara *et al.* (1985) stated that Sangiran Formation is an allochthonous sediment. Allochthonous sediment is formed by erosions of uplifted hinterland and the geological materials (such as foraminifera and diatom) were drifted and preserved in lowland (Zaim Y 15 July 2013, personal communication). Therefore, more

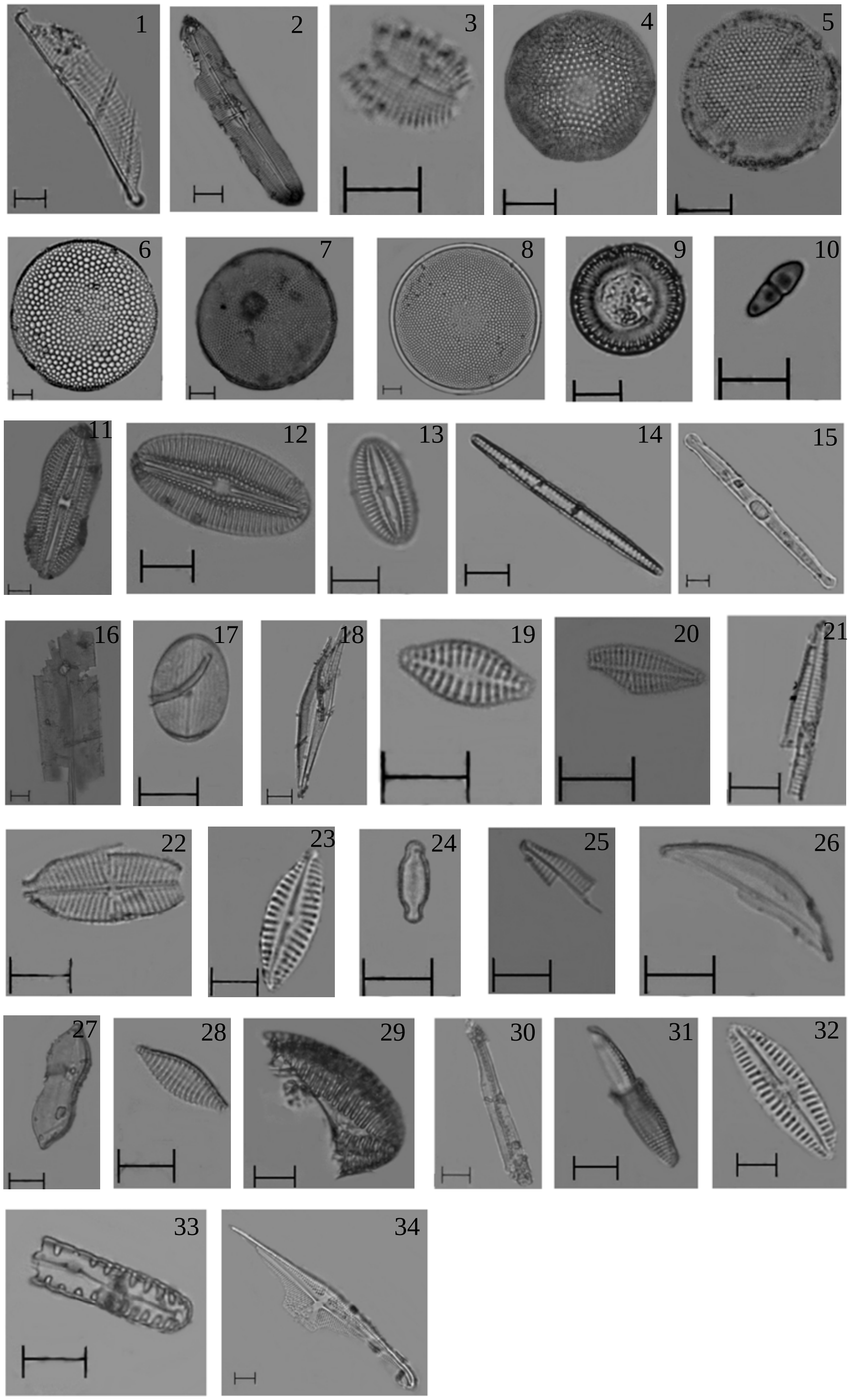

Figure 7 Alphabetical list of diatom taxa from lower diatomite layer

Remarks: 1. *Amphora* sp.; 2. *Caloneis* sp.; 3. *Cocconeis scutellum;* 4. *Coscinodiscus kutzingi;* 5. *Coscinodiscus lineatus;* 6. *Coscinodiscus radiatus;* 7. *Coscinodiscus rothii;* 8. *Coscinodiscus subtilis ;* 9. *Cyclotella* sp.; 10. *Denticula* sp.; 11. *Diploneis gemmatula;* 12. *Diploneis smithii ;* 13. *Diploneis suborbicularis;* 14. *Fragilaria* sp1.; 15. *Gramatophora merletta;* 16. *Gyrosigma balticum;* 17. *Mastogloia ovata;* 18. *Mastogloia quinquecostata;* 19. *Navicula anglica;* 20. *Navicula compressicauda;* 21. *Navicula directa;* 22. *Navicula grippii;* 23. *Navicula mannii;* 24. *Navicula* sp1.; 25. *Navicula* sp2.; 26. *Navicula* sp4.; 27. *Nitzschia acuta;* 28. *Nitzschia antartica;* 29. *Nitzschia cocconeiformis;* 30. *Nitzschia paradoxa;* 31. *Nitzschia* sp.; 32. *Pinularia yarrensis;* 33. *Progonoia* sp.; 34. *Trachyneis* sp. Scale bars = 10 μm.

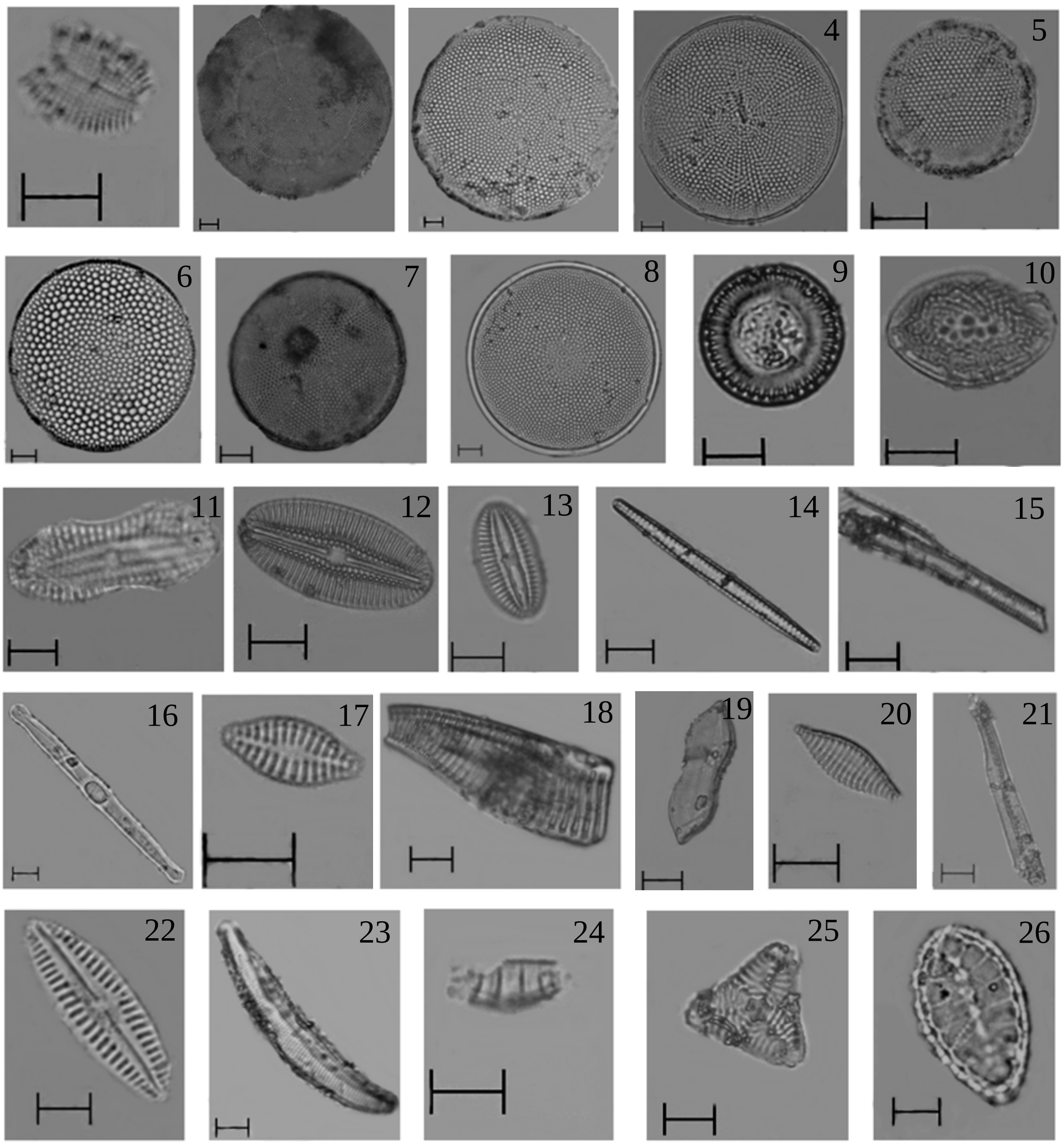

Figure 8. Alphabetical list of diatom taxa from upper diatomite layer

Remarks: 1. *Cocconeis scutellum;* 2. *Coscinodiscus biangulatus;* 3. *Coscinodiscus bulliens;* 4. *Coscinodiscus curvatulus;* 5. *Coscinodiscus lineatus;* 6. *Coscinodiscus radiatus;* 7. *Coscinodiscus rothii ;* 8. *Coscinodiscus subtilis;* 9. *Cyclotella* sp.; 10. *Cymatodiscus planetophorus;* 11. *Diploneis crabro;* 12. *Diploneis smithii;* 13. *Diploneis suborbicularis;* 14. *Fragilaria* sp1.; 15. *Fragilaria* sp2.; 16. *Gramatophora merletta ;* 17. *Navicula anglica;* 18. *Navicula* sp3.; 19. *Nitzschia acuta ;* 20. *Nitzschia antartica;* 21. *Nitzschia paradoxa ;* 22. *Pinularia yarrensis ;* 23. *Pseudoeunotia doliolus;* 24. *Rhopalodia* sp.; 25. *Schuettia annulata;* 26. *Surirella* sp. Scale bars = 10 µm.

research is needed to analyze how the erosions affecting the diatomite sedimentation.

CONCLUSION

A total of 50 species were found from diatomite layers of Sangiran. Diversity analysis showed that highest species richness, diversity, and evenness index were found in lower layer. Highest frustule abundance and dominance index were in the middle layer. However, upper layer had the highest centrales:pennales ratios. The layers showed an obvious indication of environment change from more benthic marine

SUGGESTION

More study is necessary to analyze interpretation of diversity indices for fossil diatoms. This is because most of diversity indices interpretations are of modern and live plankton. More research is also needed to analyze how erosions of uplifted hinterland affecting the diatomite sedimentation.

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