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Forensic Identification of Diatoms Based on Morphology in Major Rivers of Selangor, Malaysia

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ABSTRACT: Diatoms are photosynthesising algae containing siliceous frustule which are classified under the *Bacillariophyceae* class. Its existence are everywhere in moist environment such as rivers, ponds, lakes, soils and marine waters. There are nine morphologies of diatoms, namely centric, araphid, symmetrical biraphid, asymmetrical biraphid, nitzschioid, epithemioid, surirelloid, monoraphid and eunotioid. In forensic science, detection of diatoms in tissues may contribute to the diagnosis of drowning. This research aims to identify diatoms based on its morphology in major rivers of Selangor, Malaysia. The samples were collected by scrubbing on the boulders, pebbles and cobbles that were found to be submerged in the rivers and were facing the sunlight using a toothbrush. The rivers involved were Klang, Kemensah, Damansara, Ampang, Kanching and Gombak rivers. The collected samples of diatoms from each river were then heat fixed and observed under microscope. Diatoms were then identified by analysing the morphology of each diatom. Major rivers of Selangor have diatoms which only consisted of eight types of morphology. Diatom of surirelloid morphology was not found in Gombak, Damansara, Kemensah and Klang rivers. Diatoms of eunotioid and asymmetrical biraphid morphology were not found in Damansara and Klang River. Furthermore, diatoms of symmetrical biraphid and araphid were not found in Klang River. Centric diatoms and eunotioid diatoms were not found in Ampang River. Diatoms morphology information will help to narrow down the selection of water bodies of a particular location; linking the suspect as well as the victim. Therefore, this research is vital in forensic science as to provide information and support in the investigation for drowning site.

Keywords: diatom, drowning, Selangor's rivers

Introduction

Drowning results in suffocation following immersion of the mouth and nostrils in a liquid, thus involves aspiration of fluid into the air passage of the lung. Since diatoms contain siliceous cell wall, our body cannot easily digest them, making them to accumulate in the organs. The sign of immersion merely indicates that a body has been under water for some time. Because of diatoms can be found in many water sources, their presence can be used as an indication of drowning [1].

Diatoms are photosynthesising algae, which have siliceous skeleton frustules [2]. It can be found in almost every aquatic and moist environment including fresh and marine waters and soils. Due to their abundance in marine plankton, especially in nutrient-rich areas of the world's oceans, diatoms probably account for as much as 20% of global photosynthetic fixation of carbon which is more than the entire world's tropical rainforests [3]. More than 200 genera of living diatoms and approximately 100,000 species exists in this world [4].

The diatom belongs to Division Chrysophyta and can be divided into two orders, which are Biddulphiales (Centrales) and Pennales (Bacillariales). The classifications are based on the shape of the diatoms. The Biddulphiales usually appear radially symmetrical and Pennales appears bilaterally symmetrical. Study reported that nine basic morphology categories of diatoms were found so far [5]. The nine groups are centric, araphid, eunotioid, symmetrical biraphid, monoraphid, asymmetrical biraphid, epithemioid, nitzschioid and surirelloid [6].

Diatoms species can be found on cobbles, rocks, damp sediments facing the water surface, stems of rooted vegetation and even

on drowned victim's wet clothing. On cobbles and rocks facing the water surface, the diatoms species can be seen as a brown layer covering on top of the surfaces of the cobbles and rocks. Hence, the habitats of diatoms are rivers, lakes, ponds and seas [7].

Generally, diatoms are very sensitive to the environment that they are living in. Furthermore, some species of diatoms prefer a specific pH range and salinity. Diatoms are perceived to have different ranges and tolerances for environmental variables, such as nutrient concentration, suspended sediment, flow regime, elevation, and different types of human disturbance [8]. Since the siliceous cell walls in diatoms do not decompose, they can be used to determine the water condition in the past decades.

Diatoms have been used in forensic analysis for determining the site of drowning. Forensic analyst studies diatoms for establishing the mode (ante-mortem or post-mortem), probable season of death and also determining the putative site of drowning [9]. Therefore, in this study, the diatoms present in major rivers of Selangor were identified based on their morphology in order to provide information to support the manner of death and time since death, as well as the drowning site.

Materials and methods

Sampling

Cobbles of the diameter between 64 and 265 mm, boulders (> 256 mm diameter), and pebbles were collected from a reach of at least 10 m in the Ampang River, Damansara River, Gombak River, Kemensah River, Klang River and Kanching River.

The cobbles, boulders and pebbles were rinsed in the stream and placed in a plastic tray on the river bank, together with about 50 mL of the water body. Diatoms were removed by vigorously scrubbing the upper surface of the cobbles, boulders and pebbles with a clean toothbrush. Only the side which faces the surface of water was scrubbed to avoid contamination.

The resulting diatom suspension was then poured into a labeled wide-mouth plastic sample bottle of 100 mL capacity or greater. Finally, equipment contamination between sites was avoided by rinsing both the brush and the plastic tray in the river twice before and after taking the diatom sample.

Sample preparation

Two to three drops of fresh concentrated diatom suspension were placed onto a cover slip. Then, distilled water was added until the surface of the cover slip was almost all being covered. The cover slip was then gently heated on a hotplate until all liquid evaporated. The heat was increased to 350°C and the sample was continued to be heated until it turned white.

The coverslip was allowed to cool and removed from the hotplate. The upper surfaces of the coverslip were gently rinsed to remove any salts or other precipitates. The coverslip was the then heated again until moisture was removed from the sample.

Sample observation

Prior to microscopy examination, the sample was mounted using DPX and then gently heated again on the hotplate. The sample was viewed using Labomed CxL compound microscope fitted with a flex camera.

Results and Discussion

Many diatoms were observed in the Ampang River, Damansara River, Gombak River, Kemensah River, Klang River and Kanching River. Based on database of Diatoms of United States, the generas that can be identified are based on its morphology.

Identification of Diatoms Based on Morphology

The centric diatom as shown in Fig. 1 can be classified under the genera of Aulacoseira, Cyclostephanos, or Cyclotella. Moreover, these types of diatom are symmetric about a point. They are lacking in significant motility. The centric diatom was also identified based on its flat faces presence of striae.

Nitzschioid morphology can be identified by the presence of striae that could be more or less parallel sides, as shown in Fig. 2. This diatom can be classified under Trioblionella or Hantzschia genera. Both genera tend to exhibit lanceolate apices on both ends.

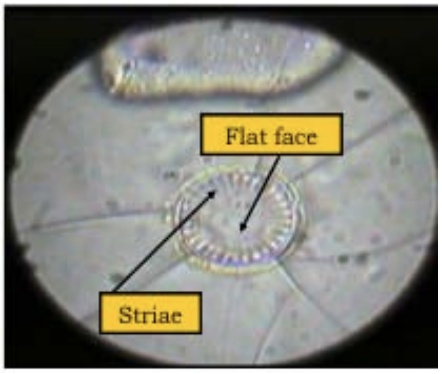

Fig. 1: Centric Morphology

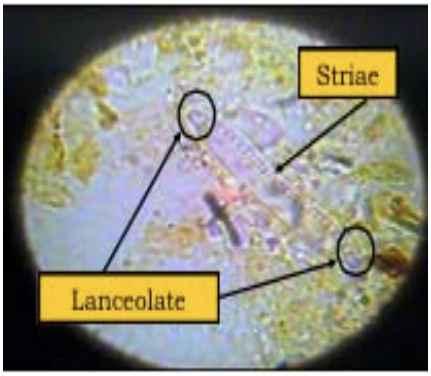

Fig. 2: Nitzschioid Morphology

Fig. 3 shows the morphology of symmetrical biraphid. This morphology can be classified under Navicula genera. It is due to presence of slightly thick striae observed under the compound microscope. The striae tend to be very short. Furthermore, it has a broad elliptical shape.

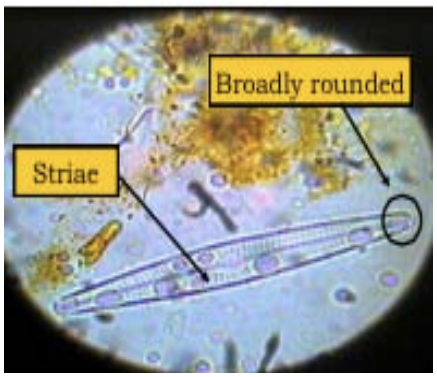

Fig. 3: Symmetrical Morphology

Diatoms that has the morphology of asymmetrical biraphid were observed in all rivers except for Klang river. It has dense striae and a half elliptical structure. It also exhibits a linear ventral margin. As shown in Fig. 4, both of the apices are broadly rounded. This diatom can be classified under Encyonema genera.

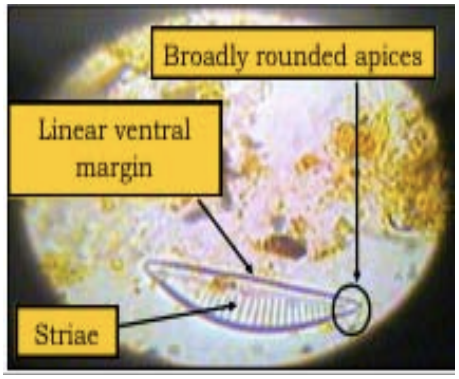

Fig. 4: Asymmetrical Morphology

The presence of striae with abrupt striations can be observed under the compound microscope. The striae are very dense. The morphology can be identified as monoraphid diatom, Fig. 5. This diatom, which is motile, can be classified under Achnanthes genera.

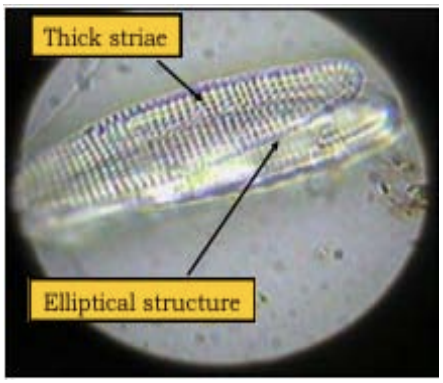

Fig. 5: MonoraphidMorphology

Fig. 6 shows a diatom of Eunotia genera under Eunotioid morphology. The Eunotioid morphology can also be found in Selangor's major rivers except for Klang River, Damansara River and Ampang River. This is due to the morphology of Eunotioid which have a slightly rectangular shape. The shape changes when it is still in growing stage. Eunotioid diatoms have parallel striae and also weak motility.

Fig. 7 shows a diatom consist of Araphid morphology. This morphology has parallel striae and thin elongating structure. It also has broadly rounded apices and exhibits poor motility. It is classified under genera of Fragilaria.

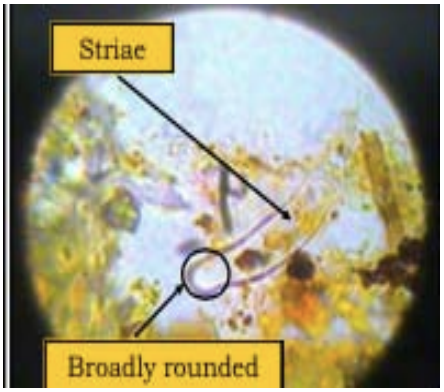

Fig. 6: Eunotioid Diatom

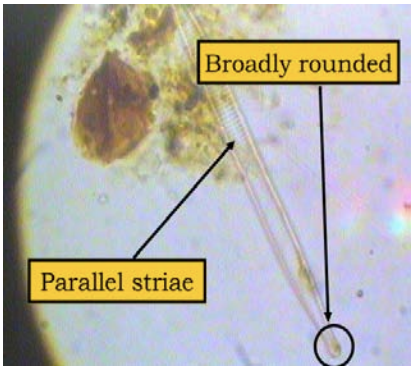

Fig. 7: Araphid Morphology

Determination of Diatoms in Selangor's River

Eight morphologies of diatoms found in Selangor's major rivers identified in this study are Centric, Nitzschioid, Symmetrical Biraphid, Asymmetrical Biraphid, Surireloid, Monorophid, Eunotioid and Araphid as in Table 1.

Nitzschioid and Monorophid morphologies of diatoms were present in all rivers. Symmetrical Biraphid was not present in the Klang River. Surirelloid morphology present in some rivers such as Ampang, Kanching and Gombak rivers. Eunotioid morphology could only found in Kemensah, Kanching and Gombak Rivers. The location of Symmetrical and Asymmetrical diatoms was different, where the Asymmetrical diatoms were found in four rivers (Kemensah, Ampang, Kanching and Gombak rivers), whereas symmetrical diatoms were found in Damansara River.

Table 1: Type of Diatom Morphologies Present in Major Rivers of Selangor

Types of Morphologies	Location of rivers					
	Klang River	Kemensah River	Damansara River	Ampang River	Kanching River	Gombak River
Nitzschioid	X	X	X	X	X	X
Centric	X	X	X		X	X
Araphid		X	X	X	X	X
Symmetrical Biraphid		X	X	X	X	X
Asymmetrical Biraphid		X		X	X	X
Surirelloid				X	X	X
Monorophid	X	X	X	X	X	X
Eunotioid		X			X	X

Less variation in morphology of diatoms was seen in water from three types of diatom morphologies (Nitzschioid, Centric and Monorophid) were found. On the contrary, Kanching River and Gombak water consisted of all types of diatoms based on its basic eight morphologies. This could be due to comparatively clean water body in these rivers compared to a polluted river such as the Klang river.

Conclusion

Diatoms found in various rivers in Selangor were identified based on their various morphologies, including Centric, Araphid, Eunotioid, Symmetrical Biraphid, Monoraphid, Asymmetrical Biraphid, Nitzschioid, Epithemioid and Surirelloid. Nitzschioid and Monorophid diatoms were present in all six rivers. Klang River consisted of only three types of diatoms, namely Nitzschioid, Centric and Monorophid, partly because the water was polluted. The diatoms discovered in water could be used to establish a link between the putative drowning medium and the drowned victim, thus act as secondary supportive evidence in suspicious drowning cases, especially when the morphologies vary in different rivers.

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