

Studies on Diatom Diversity of Various Water Bodies of The State Tripura (India) for Forensic Analysis

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How to cite this article: Sabyasachi Nath, Subhechha Satapathy. Studies on Diatom Diversity of Various Water Bodies of The State Tripura (India) for Forensic Analysis. Indian Journal of Forensic Medicine & Toxicology / Vol 20 No. 1, January - March 2026

Abstract

Diatoms are unicellular algae with unique silica frustules found in diverse aquatic and terrestrial ecosystems. In forensic science, their presence in distant vital organs aids in determining whether drowning occurred ante-mortem and helps identify the possible site of drowning. In Tripura, a small hilly state in the north-east India, there is a significant annual incidence of drowning cases with no existing diatom database. A recent study conducted across four out of eight districts of Tripura aims to establish a comprehensive diatomological database to aid forensic experts in drowning investigations. The study identified 73 species of diatoms belonging to 34 genera and 22 families under 11 orders of the class Bacillariophyceae across different districts with some species common among all districts, while others are exclusive to specific regions showcasing a diverse range of diatoms. Among them, there are 11 species from both the Bacillariaceae and Naviculaceae families, 8 from Gomphonemataceae, 7 from Fragilariaceae, 5 from Pinnulariaceae, 4 each from Achnanthidiaceae and Cymbellaceae, 3 from Sellaphoraceae along with 2 species each from Aulacoseiraceae, Catenulaceae, Diadesmidaceae, Pleurosigmataceae, Stephanodiscaceae and Rhopalodiaceae and 1 species each from the families Achnanthaceae, Brachysiraceae, Eunotiaceae, Mastogloiaceae, Melosiraceae, Neidiaceae, Stauroneidaceae and Surirellaceae. The West district recorded the highest diversity with 36 species, followed by Sepahijala with 30 species, Unakoti with 16 and Gomati with 14 species. Interestingly two species are of common occurrence among all the four districts, while some are exclusive to certain regions. Twenty-two species were exclusively found in the West district, where as 17 species were restricted only to the Sepahijala district. Likewise, 10 species were limited to the Unakoti district and 7 species were found solely in the Gomati district. Certain species are unique to specific region underscoring notable variations in diatom distribution across different water bodies. Moreover, this study adds 72 species to the previously recorded 21 species of diatoms in Tripura. The diverse distribution of diatoms across water bodies aids in linking drowned individuals to specific sites helping identify drowning locations and solving cases.

Key words: Diatom, Drowning sites, Silica frustule, D-Map, Diatom diversity, Diatom database.

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Submission: Oct 31, 2025

Revision: November 10, 2025

Published date: January 29, 2026

Introduction

Diatoms, the microscopic, unicellular algae classified under Bacillariophyceae of the kingdom Protista, are known for their silica-based frustules which display intricate patterns earning them the title 'jewels of the plant world'. A study indicates that over 200 genera and approximately 200,000 diatom taxa exist worldwide¹. In the Indian subcontinent, around 14,700 taxa of diatoms are estimated including freshwater, brackish and marine varieties². They occur in diverse aquatic habitats such as rivers, lakes, ponds, brackish and marine waters as well as in terrestrial environments such as mosses, wet rocks and soil^{3,4,5}. Diatoms contribute about 25% of atmospheric oxygen and 43% of oceanic primary production significantly influencing global carbon cycling⁶.

Diatoms exhibit a remarkable diversity in shapes and sizes ranging from circular to elongated and even triangular forms with dimensions typically between 2µm and 2mm, most commonly 10-80µm. In freshwater ecosystems, they act as key primary producers and serving as food for zooplankton and small fishes. They are also recognized as reliable bio-indicators for assessing water quality due to their environmental sensitivity. Moreover, their silica frustules have forensic significance especially in drowning investigations for determining drowning type, location, time since death and evidence of asphyxia.

Diatom communities vary widely among water bodies due to differences in nutrient and light availability with factors such as temperature, pH, mineral content and water stratification influencing their abundance and diversity^{7,8,9,10}. Studies have shown that low pH reduces diatom richness, whereas circum-neutral pH supports higher species richness and diversity¹¹. Research also highlights the forensic significance of diatoms as distinct freshwater and marine species help determine drowning sites. Diatom testing in organs and bone marrow aids such investigations. Some studies in France and Delhi revealed site-specific and seasonal variations respectively, assisting in drowning analysis^{12,13}.

Diatoms were first recognized in lung fluids by Hoffman in 1896, but their forensic relevance emerged when Hoffman and Rovenstorff used them as evidence in a drowning case in 1904. Later, Incze (1942) detected diatoms in blood and organs and Tamasaka (1949) in bone marrow. In India, Ehrenberg first reported diatoms from the Nilgiris mountains, Ganges and Southern coasts in 1845 using light microscopy². Pioneering studies on freshwater diatoms were conducted by H.P. Gandhi¹⁵, Sarode and Kamat¹⁶. Between 2011 and 2012, many studies have been made on diatom diversity; Pareek *et al* studied 24 diatom species in Galta Kund, Jaipur, Huifang Su *et al* examined the Pearl and Yangtze rivers (China), Marbaniang and Paul identified 22 species in Meghalaya, Singh *et al* reported 22 genera from the Ganga, and Saini and Kushwaha found 39 species in Haryana including 6 new species^{17,18,19,20,21,22,23,24}, while Gupta *et al*, Luthra *et al*, Chawla and Patel, and Akhila *et al* explored Himachal Pradesh, Punjab, Madhya Pradesh and Andhra Pradesh revealing ecological and forensic significance of diatoms^{25,26,27,28}. Diatomological maps could potentially identify drowning sites when locations are unclear as supported by various researchers^{8,29,30,31,32,33}. Thakur and Singh created a Diatomological map of Punjab's water bodies to assist drowning investigations³⁴. Singh *et al* created a diatom database for Jaipur identifying rare site-specific diatoms³⁵.

In Tripura, a small hilly state in the north-east India, with numerous drowning incidents annually, there is currently no comprehensive diatom database for comparison with tissue samples from victims. Although Das *et al*³⁶ studied algae in Tripura from three subdivisions, a thorough investigation of diatom diversity across all eight districts remains pending. This study aims to survey at least two water bodies from each of four districts namely West Tripura, Sepahijala Tripura, Unakoti Tripura and Gomati Tripura districts and to establish a foundational database for future research on drowning cases.

Materials and Methods

Study Area

A survey was conducted during January-July, 2024 in four districts of Tripura (22°51'-24°32'N, 90°10'-92°21'E; area:10,491sq.km) namely West,

Sepahijala, Gomati and Unakoti districts (Figure 1). The state experiences summer (April-June), monsoon (July-October), winter (November-February) and a brief spring (March) with an average annual rainfall of approximately 2000mm.

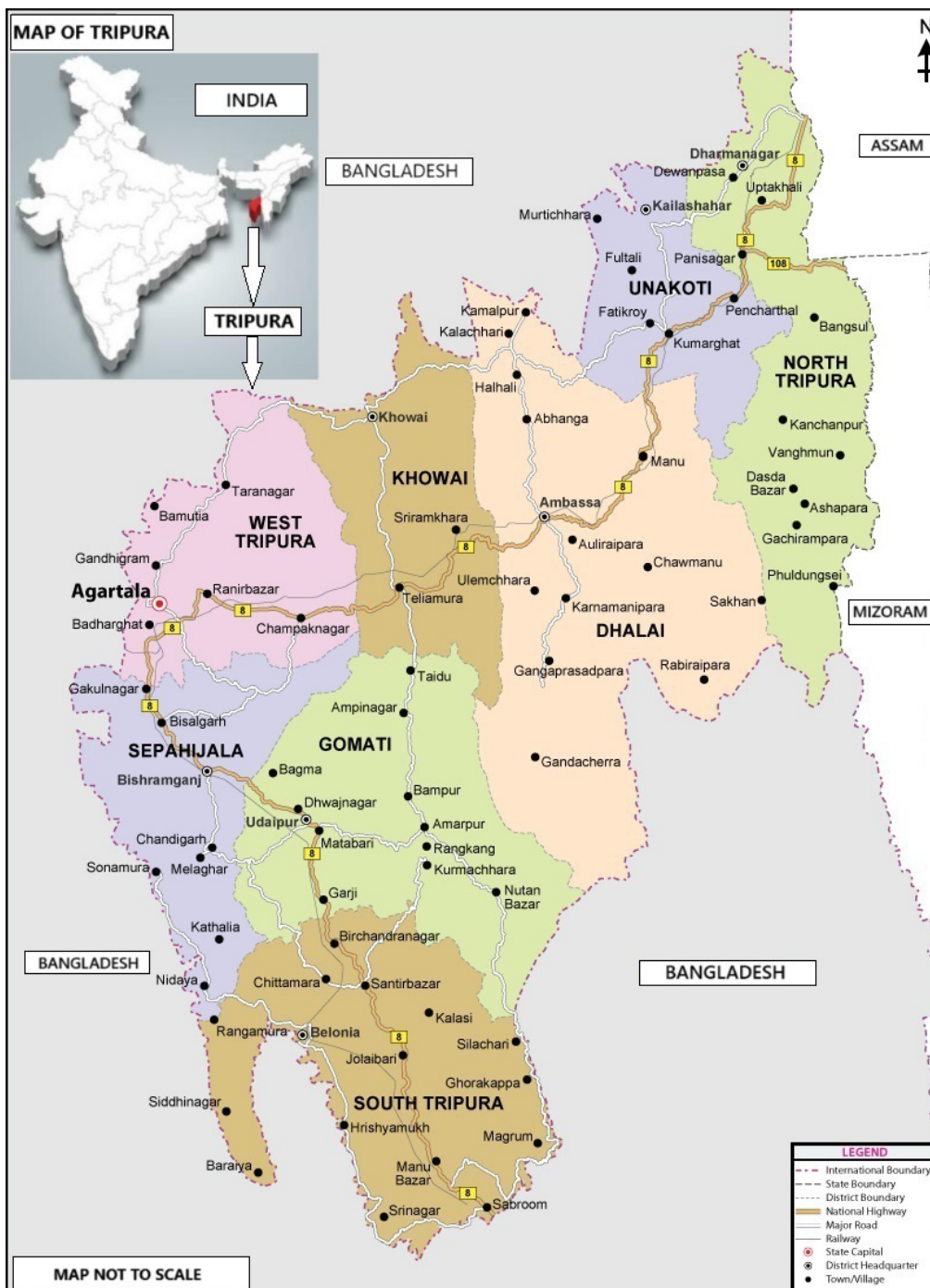


Figure 1: Map of Tripura showing its eight districts [courtesy: www.mapsofindia.com].

Study Sites

Study sites selected for diatom survey include two types of water bodies-ponds and lakes, across four districts in Tripura. A minimum of two water bodies were selected from each district for this study.

Sample Collection

Approximately 100ml of water was collected in clean plastic bottles by filtering 30 liters of water through a 20 μ m mesh plankton net at four sites per pond/lake. The bottles were sealed and labeled with site, date and time, and were transported to the laboratory and refrigerated.

Sample Preparation

In a 100 ml water sample, 2-3 drops of 2% formalin were added and was left overnight. Half of the sample was then discarded and the remaining portion was vigorously shaken and centrifuged at 3000rpm for 10minutes. The supernatant was discarded reducing the volume by half. Approximately 10-12ml of the residual pellet was transferred to a test tube, treated with 2ml of 50% H₂O₂ and 5ml of concentrated HNO₃ and left undisturbed for about six hours for digestion. The digested sample was transferred to a labeled centrifuge tube, centrifuged again at 3000rpm for 10minutes and washed thrice with distilled water to eliminate residual acids. Finally, two drops of distilled water were added to the pellet, stored at room temperature and one drop was mounted on a slide using DPX for microscopic examination^{35,37}. The slides were observed under a compound microscope at varying magnifications (100X, 400X, 1000X) and the images were captured.

Identification of Diatoms

The diatoms were identified through a review of existing literature including resources such as works by Charles E. Bessey³⁸, the online database by P.A. Sims³⁹ and the database compiled by Karthick *et al*². Their identification was based on various morphological characteristics.

Results and Discussion

A total of 73 species of diatoms were collected from four of the eight districts in Tripura, representing 34 genera and 22 families under 11 orders of the class Bacillariophyceae of the kingdom Protista (Table 1). Among these, there are 11 species each from the families Bacillariaceae (*Bacillaria paradoxa*, *Denticula* cf. *kuetzingii*, *Nitzschia agnita*, *N. amphibia*, *N. gracilis*, *N. hantzschia*, *N. intermedia*, *N. palea*, *N. cf. pura*, *N. taylorii*, *Hantzschia amphioxys*) and Naviculaceae (*Navicula angusta*, *N. arenaria*, *N. cryptocephala*, *N. cryptotenella*, *N. erifuga*, *N. heimansioides*, *N. cf. leistikowii*, *N. notha*, *N. cf. radiosa*, *N. rostellata*, *N. schroeteri*). Additionally, there are 8 species from Gomphonemataceae (*Gomphonema affine*, *G. angustatum*, *G. exilissimum*, *G. lagenula*, *G. parvulum*, *G. pseudoaugur*, *G. pseudosphaerophorum*, *Gomphonema* sp), 7 from Fragilariaceae (*Diatoma vulgare*, *Fragilaria crotonensis*, *Pseudostaurosira tenuis*, *Ulnaria acus*, *U. delicatissima*, *U. ulna*, *Ulnaria* sp), 5 from Pinnulariaceae (*Caloneis aequatorialis*, *Pinnularia acrosphaeria*, *P. amabilis*, *P. viridis* and *Pinnularia* sp), 4 species each from Achnanthidiaceae (*Achnanthidium minutissimum*, *A. saphophilum*, *Achnanthidium* sp, *Planothidium rostratum*) and Cymbellaceae (*Cymbella kolbei*, *C. parva*, *Oricymba japonica*, *Placoneis molestissima*) and 3 species from Sellaphoraceae (*Sellaphora alastos*, *S. bacillum*, *Sellaphora* sp). Moreover, 2 species each belong to the families Aulacoseiraceae (*Aulacoseira herzogii*, *A. nivaloides*), Catenulaceae (*Amphora copulate*, *A. pediculus*), Diadesmidaceae (*Luticola* cf. *kotschyi*, *L. mutica*), Pleurosigmaaceae (*Gyrosigma eximium*, *Pleurosigma* sp), Stephanodiscaceae (*Cyclotella atomus*, *Cyclotella* sp) and Rhopalodiaceae (*Epithemia adnata*, *Rhopalodia musculus*). Lastly, there is 1 species each from the families Achnanthaceae (*Lemnicola hungarica*), Brachysiraceae (*Brachysira microcephala*), Eunotiaceae (*Eunotia minor*), Mastogloiaceae (*Mastogloia smithii*), Melosiraceae (*Melosira varians*), Neidiaceae (*Neidium productum*), Stauroneidaceae (*Stauroneis acuta*) and Surirellaceae (*Surirella capronioides*). Among the diatom species recorded, 36 species were found in the water bodies of West district, followed by 30 species in Sepahijala district, 16 species in

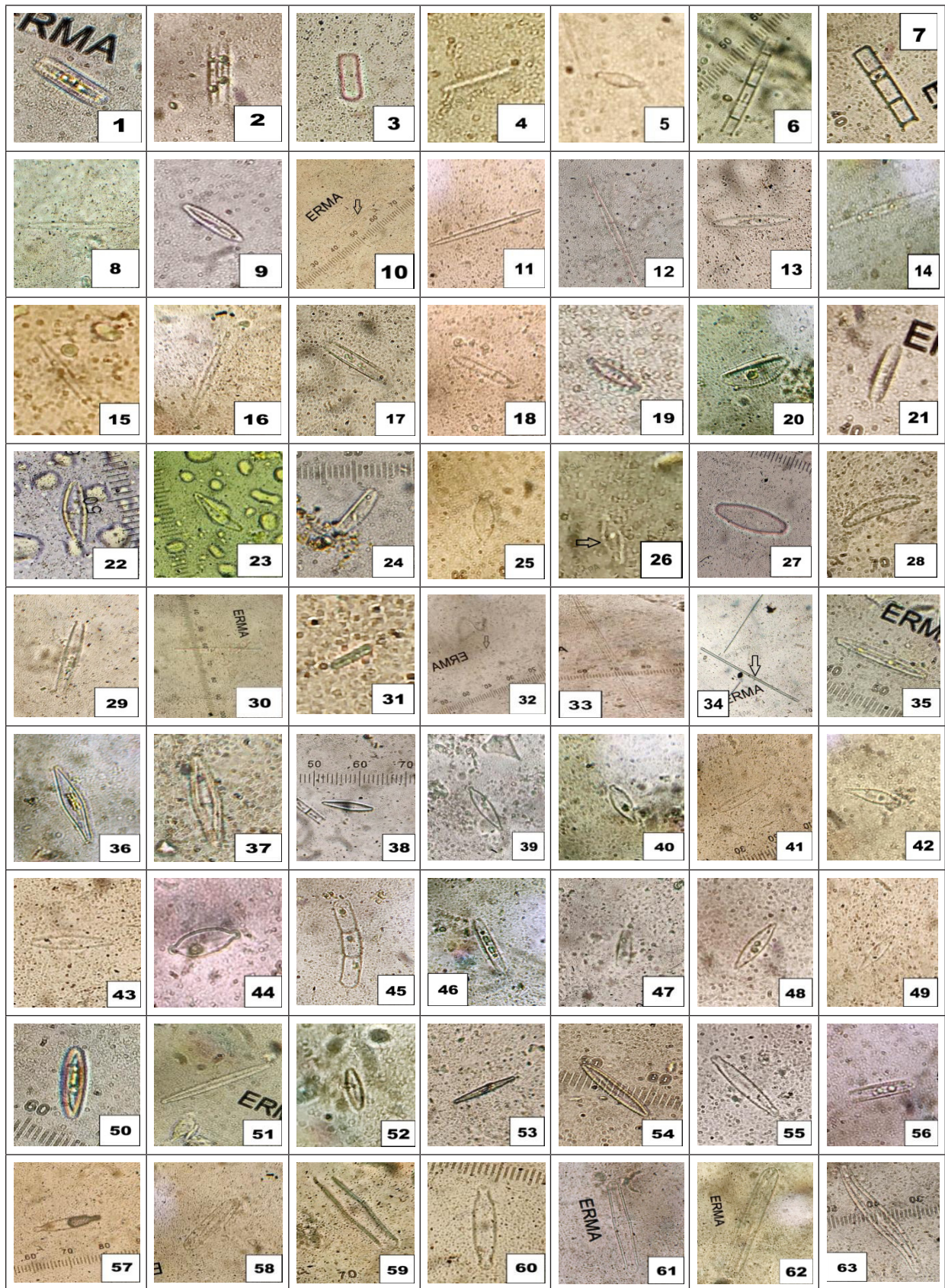
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53	<i>Navicula notha</i> Wallace	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
54	<i>Navicula cf. radiosa</i> Kützing	-	+	-	+	-	-	+	+	-	-	-	+	-	-	-
55	<i>Navicula rostellata</i> Kützing	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-
56	<i>Navicula schroeteri</i> Meister	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
57	O. NEIDIACEAE <i>Neidium productum</i> (Smith) Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	+	-
58	P. PINNULARIACEAE <i>Caloneis aequatorialis</i> Hustedt	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
59	<i>Pinnularia acrosphaeria</i> Smith	-	-	-	-	-	-	-	-	+	-	+	-	-	-	-
60	<i>Pinnularia amabilis</i> Krammer	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
61	<i>Pinnularia</i> sp	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
62	<i>Pinnularia viridis</i> (Nitzsch) Ehrenberg	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
63	Q. PLEUROSIGMATACEAE <i>Gyrosigma eximium</i> (Thwaites) Boyer	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
64	<i>Pleurosigma</i> sp	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
65	R. RHOPALODIACEAE <i>Rhopalodia musculus</i> (Kützing) Müller	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
66	<i>Epithemia adnata</i> (Kützing) Brébisson	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
67	S. SELLAPHORACEAE <i>Sellaphora alastos</i> (Hohn & Hellerman) Lange-Bertalot & Metzeltin	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
68	<i>Sellaphora bacillum</i> var. <i>jogensis</i> Gandhi	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
69	<i>Sellaphora</i> sp	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-
70	T. STAURONEIDACEAE <i>Stauroneis acuta</i> Smith	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-
71	U. STEPHANODISCACEAE <i>Cyclotella atomus</i> Hustedt	-	-	+	-	-	-	-	-	-	-	-	+	-	-	-
72	<i>Cyclotella</i> sp	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
73	V. SURIRELLACEAE <i>Surirella capronioides</i> Gandhi	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+

‘+’ = present; ‘-’ = absent.

Distribution and abundance of diatoms are influenced by a number of physical and biological factors. In different water bodies in the present study, diatoms experienced water temperature ranging from 21.5-36.0°C and a pH from 5.0-7.0. Das *et al* made some studies on algae of Tripura where

they found 21 species of diatoms³⁶. Of those, only one species, *i.e.*, *Nitzschia intermedia* Hantzsch, is common to the 73 species found in the present study (Figure 2) thus contributing 72 more species to the previous record. Thus a total of 93 species have been identified in the state to date.



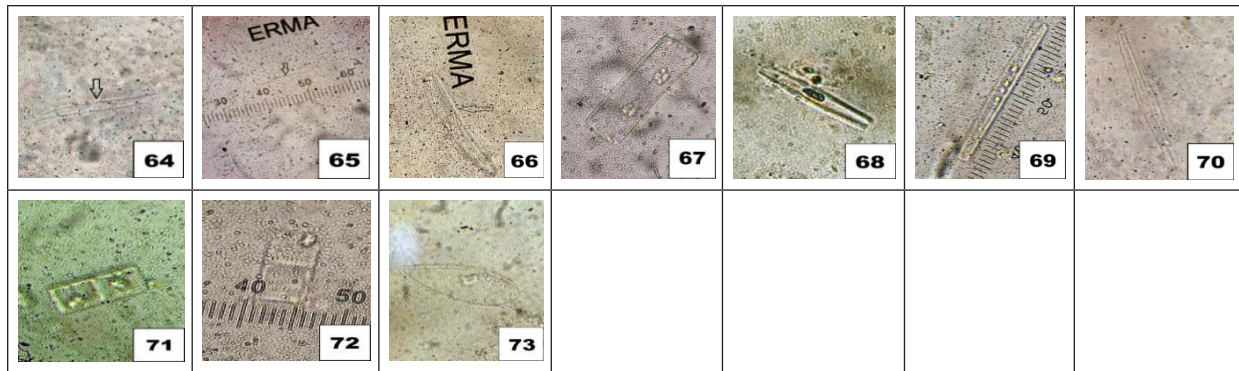


Figure 2: Diverse diatom species identified in various water bodies across four districts of the state Tripura: (1) *Lemnicola hungarica* (Grunow) Round et Basson, (2) *Achnantheidium minutissimum* (Kützing) Czarnecki, (3) *Achnantheidium saprophyllum* (Kobayasi & Mayama) Round & Bukhtiyarova, (4) *Achnantheidium* sp, (5) *Planothidium rostratum* (Østrup) Lange-Bertalot, (6) *Aulacoseira herzogii*, (7) *Aulacoseira nivaloides*, (8) *Bacillaria paradoxa* Gmelin, (9) *Denticula* cf. *kuetzingii* Grunow, (10) *Nitzschia agnita* Hustedt, (11) *Nitzschia amphibia* Grunow, (12) *Nitzschia gracilis* Hantzsch, (13) *Nitzschia hantzschia* Rabenhorst, (14) *Nitzschia intermedia* Hantzsch, (15) *Nitzschia palea* (Kützing) Smith, (16) *Nitzschia* cf. *pura* Hustedt, (17) *Nitzschia taylorii* Alakananda, Hamilton & Karthick, (18) *Hantzschia amphioxys* (Ehrenberg) Grunow, (19) *Brachysira microcephala* (Grunow) Compère, (20) *Amphora copulate* (Kützing) Schoeman & Archibald, (21) *Amphora pediculus* (Kützing) Grunow, (22) *Cymbella kolbei* Hustedt, (23) *Cymbella parva* (Smith) Wolle, (24) *Oricymba japonica* (Reichelt) Jüttner, Cox, Krammer & Tuji, (25) *Placoneis molestissima* Metzeltin, Lange-Bertalot & Garcia-Rodriguez, (26) *Luticola* cf. *kotschy* Grunow, (27) *Luticola mutica* (Kützing) D.G. Mann, (28) *Eunotia minor* (Kützing) Grunow, (29) *Diatoma vulgare* Bory, (30) *Fragilaria crotonensis* Kitton, (31) *Pseudostaurosira tenuis* Morales & Edlund, (32) *Ulnaria acus* (Kützing) Aboal, (33) *Ulnaria delicatissima* (Smith) Aboal & Silva, (34) *Ulnaria* sp, (35) *Ulnaria ulna* (Nitzsch) Compère, (36) *Gomphonema affine* Kützing, (37) *Gomphonema angustatum* (Kützing) Rabenhorst, (38) *Gomphonema exilissimum* (Grunow) Lange-Bertalot & Reichardt, (39) *Gomphonema lagenula*

(Kützing) (40) *Gomphonema parvulum* (Kützing) Kützing sensu strict, (41) *Gomphonema pseudoaugur* Krammer, (42) *Gomphonema pseudosphaerophorum* Ehrenberg, (43) *Gomphonema* sp, (44) *Mastogloia smithii* Thwaites, (45) *Melosira varians* Agardh, (46) *Navicula angusta* Grunow, (47) *Navicula arenaria* Lange-Bertalot, (48) *Navicula cryptocephala* Kützing, (49) *Navicula cryptotenella* Lange-Bertalot, (50) *Navicula erifuga* (Müller) Bory, (51) *Navicula heimansioides* Lange-Bertalot, (52) *Navicula* cf. *leistikowii* Lange-Bertalot, (53) *Navicula notha* Wallace, (54) *Navicula* cf. *radiosa* Kützing, (55) *Navicula rostellata* Kützing, (56) *Navicula schroeteri* Meister, (57) *Neidium productum* (Smith) Hustedt, (58) *Caloneis aequatorialis* Hustedt, (59) *Pinnularia acrosphaeria* Smith, (60) *Pinnularia amabilis* Krammer, (61) *Pinnularia* sp, (62) *Pinnularia viridis* (Nitzsch) Ehrenberg, (63) *Gyrosigma eximium* (Thwaites) Boyer, (64) *Pleurosigma* sp, (65) *Rhopalodia musculus* (Kützing) Müller, (66) *Epithemia adnata* (Kützing) Brébisson, (67) *Sellaphora alastos* (Hohn & Helleman) Lange-Bertalot & Metzeltin, (68) *Sellaphora bacillum* var. *jogensis* Gandhi, (69) *Sellaphora* sp, (70) *Stauroneis acuta* Smith, (71) *Cyclotella atomus* Hustedt, (72) *Cyclotella* sp, and (73) *Surirella capronioides* Gandhi.

Diatomological Maps provide valuable information on the presence and distribution of common, seasonal, rare and site-specific diatom species. By presenting qualitative and quantitative data across different seasons for specific water bodies, D-Maps greatly assist forensic scientists in identifying drowning sites making their creation crucial for targeted water body investigations.

Conclusion

A comprehensive study of diatom diversity across four districts of Tripura revealed 73 species, expanding the state's known diatom record from 21 to 93 species with an addition of 72 new ones. About 70% of the species exhibited site-specific distribution with *Amphora copulata* and *Gomphonema parvulum* found ubiquitously across all districts, while 56 species showed district-wise confinement making them effective hydrological markers. The significant diatom diversity across habitats highlight Tripura's understudied aquatic ecosystems as reservoirs of microbial diversity and provides a framework for forensic limnology applications. The study established a foundational diatomological database for forensic use in drowning investigations providing reference data for linking diatom assemblages in body tissues to potential drowning sites. Future objectives include expanding sampling to all districts, continuous seasonal monitoring and developing a state-wide diatomological map to enhance medico-legal investigations and align with global forensic standards.

Conflicts of Interest Statement: The authors declare no conflict of interest in this article.

- **Ethical Clearance:** Since no human cadaver or no particular case study or no animal or no ethical issue is involved in this case, no ethical clearance is sought.
- **Funding Sources if Applicable:** No
- **Acknowledgements:** The second author expresses her sincere gratitude to the Director of the State Forensic Science Laboratory, Tripura, for granting her permission and providing the necessary facilities to conduct this research. The support and resources made available were instrumental in the successful completion of this study.

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