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DIATOM DIVERSITY IN SOME HOT SPRINGS OF NORTHERN THAILAND

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Abstract

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Diversity of hot spring diatoms in northern Thailand was studied. Forty-six diatom species were identified in eight localities. The dominant species according to high relative abundance were *Diatomella balfouriana* (41.7%), *Achnanthidium exiguum* (20.9%) and *Anomoeoneis sphaerophora* (11.2%). Moreover, *Caloneis molaris*, *Craticula acidoclinata*, *Navicula subrhynchocephala* and *Pinnularia saprophila* were recorded as species new to Thailand. The NMDS ordination revealed variation in species composition of eight different hot springs and correlation with the existing environmental variables. Silicon dioxide (SiO_2), pH, conductivity, water temperature and total hardness were statistically significant factors affecting relative abundance of *Achnanthidium exiguum*, *Amphora montana*, *Caloneis aequatorialis*, *Cocconeis placentula*, *Craticula cuspidata*, *Diploneis elliptica*, *Gomphonema affine*, *Gomphonema augur*, *Halamphora fontinalis*, *Planothidium lanceolatum*, *Pinnularia abaujensis*, *Sellaphora lanceolata* and *Stauroneis anceps*.

Keywords: bacillariophyta, extreme habitat, physicochemical factors, thermal springs.

INTRODUCTION

Diatoms are important microorganisms present at the beginning of the food chain in aquatic ecosystems. In fact, diatoms can grow in extreme habitats such as hot springs that have high temperatures and differing physicochemical characteristics from other ecosystems. The study of diatoms in such habitats may lead to the discovery of potent diatoms that can be used in various heat-tolerant applications (Pruetiworanan et al., 2017), such as diatoms that can be grown at high temperatures and this would allow researchers to make use of enormous un-used tracks of desert land for biofuel production (Gendy & El-Temtamy, 2013). Therefore, it is important to study the biodiversity database of hot spring diatoms. In extreme ecosystems, diatoms are present in both their benthic and planktonic forms (Hynes, 1970). The distribution of benthic diatoms is usually found in high abundance on rocky and cobble substrates; however, they are also known to inhabit epiphytic and epipsammic communities. Diatom distribution is distinguished by environmental characteristics that limit species distribution and the scope is restricted by the capabilities of species. The specific arrangement of diatoms can also broaden according to the relevant environmental factors (Kristiansen, 1996). Diatom species can grow in places experiencing a wide range of temperatures from 10°C to 45°C, which can occur in extreme habitats (Round et al., 1990). This temperature range involves higher temperatures when compared to the growth ability of other green algae (Nurul et al., 2013).

Numerous hot springs have been discovered throughout northern Thailand (Singharajwarapan et al., 2012). The temperatures of those thermal springs are within the range of 45–100°C, with a general flow-rate of less than 1 L s^{-1}. The pH values of the hot springs ranged from 6.5 to 9.5. Overall, the hot spring waters contain small amounts of chemicals, and also have low total dissolved solids (TDS) and gas contents (CHUAVIROJ, 1988). Most of the hot springs in Thailand are located in national parks and many of these have been recently promoted for the purposes of tourism. However, the tourist activities may negatively affect the ecosystem and lead to a loss of biodiversity. Previous research studies have reported on the cyanobacteria community in hot springs throughout northern Thailand (SOMPONG et al., 2005); however, studies on the diversity of hot spring diatoms in Thailand have not been conducted yet. Consequently, the main objective of this study was to survey the current taxonomic composition of hot spring diatoms in northern Thailand and evaluate their relationships with any relevant environmental variables. The knowledge obtained from this research will be used to create a database that can be employed to evaluate the effects of tourism on the diversity of diatoms and will also be the basis of a reference resource for the screening and application of these hot spring diatoms in the future.

MATERIALS AND METHODS

Location and description of samples sites

Eight hot springs in northern Thailand possessing differing geological characteristics were chosen for this study (Fig. 1). These chosen hot springs represent the hot springs scattered throughout northern Thailand (Fig. 2). Samples were collected once in each locality from December 2015 to April 2016. Environmental data of each sampling site were recorded (Table 1).

Water sampling analysis

To obtain the physical-chemical factors according to the method of GREENBERG et al. (1992), physicochemical parameters, particularly pH, conductivity, NO_3^-, NH_4^+, SRP, SiO_2, S^{2-}, total hardness, alkalinity and water temperature, were measured at the sampling sites (Table 2). Three replicates of water samples from each point were collected. Water samples were collected in polyethylene bottles and kept in a thermal box to retain the appropriate temperature.

Investigation of benthic diatoms

The periphytic (epipelic and epilithic) diatom samples were collected (according to the method of ROTT et al. (1997)) from the stones or the border of the hot spring pools, three replicates from each range of temperature. A plastic sheet (10 cm^2) was placed on the upper surface of the selected substrates. Each sample was collected into a small plastic container labelled with the site name, location code, date and replicate number. All samples were kept in a storage box to retain the original temperature. The procedure for diatom sample digestion and cleaning using 70% nitric acid was employed at the laboratory. These samples were mounted with a mounting agent (Pleurax) on glass slides and covered with coverslips. Then toluene in the slide was removed by being heated on a hot plate.

Benthic diatom identification and counting

A light microscope was used to identify the diatom samples, with the magnification 40× and 100×. The specimens were photographed using Olympus Nomarski microscope and scanning electron microscope. The samples were identified according to relevant determination literature; FOGED (1974; 1979); KRAMMER & LANGE-BERTALOT (1991a, b); LANGE-BERTALOT & KRAMMER (1989); LANGE-BERTALOT (2001), KRAMMER (1997a, b); KRAMMER (2000); KRAMMER (2002); KRAMMER (2003); KELLY & HAWORTH (2002) and JÜTTNER et al. (2011).

Statistical analysis

The diversity of benthic diatoms was quantified by Shannon Weiner's Diversity Index (SHANNON, 1948). The ensuing formula was used to calculate the values:

$$H' = -\sum_{i=1}^{R} p_i \ln p_i$$

where H′ = Shannon Weiner's Diversity Index,
pi = ratio of individuals in the *i*thspecies,
R = number of species

$$E = \frac{H'}{\ln S}$$

where E = evenness, S= total number of species in the population.

Diatom assemblage patterns were examined utilizing several approaches including R Project for

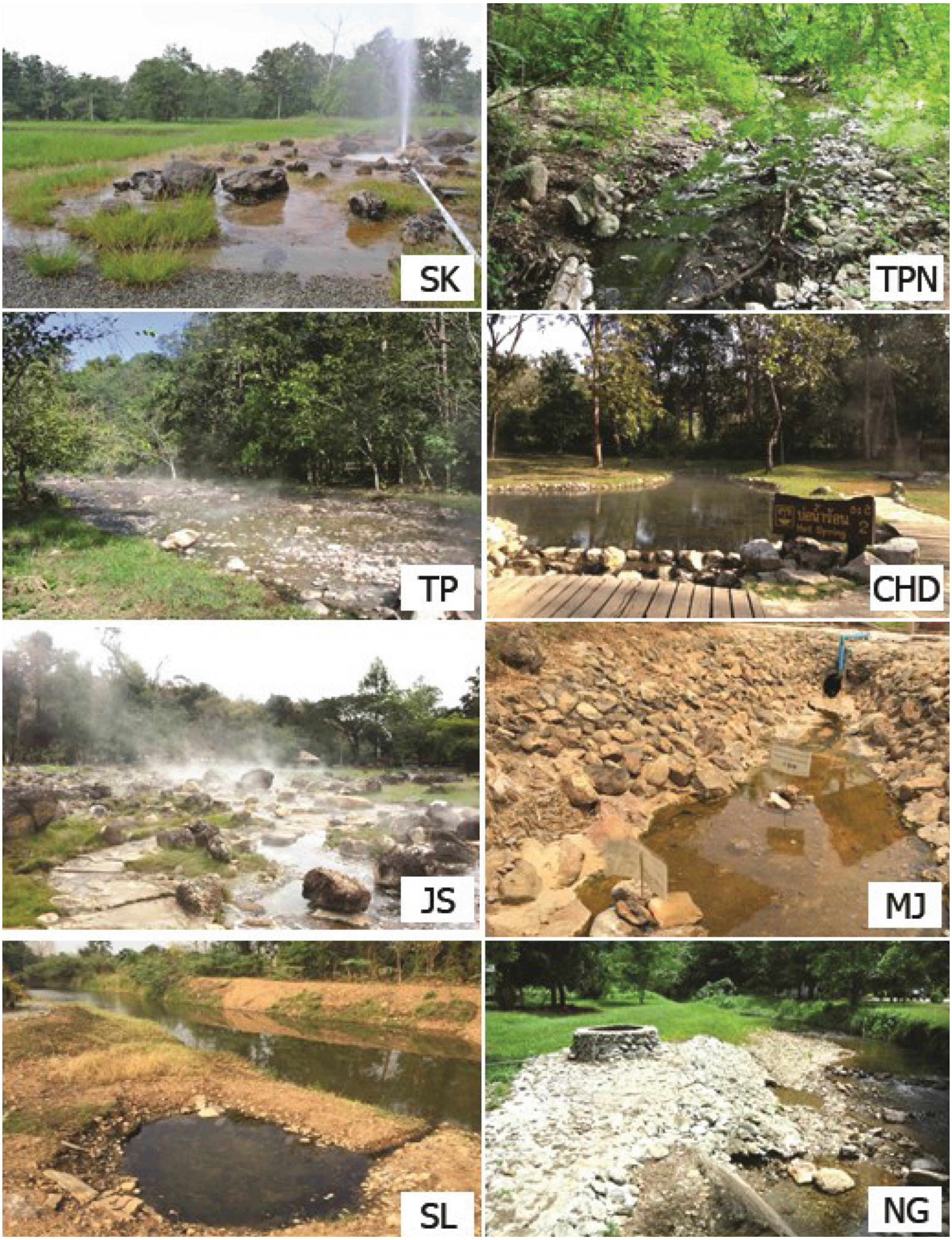

Fig. 1. Photos of eight hot springs in northern Thailand: SK – San Kamphaeng hot spring, Chiang Mai province; TPN – Theppanom hot spring, Chiang Mai province; TP – Ta Pai hot spring, Mae Hong Son province; CHD – Pong Ang hot spring, Chiang Mai province; JS – Chae Sorn hot spring, Lampang province; MJ – Mae Chok hot spring, Phrae province; SL – Wat Salaeng hot spring, Phrae province; NG – Pong Gi hot spring, Nan province (Pruetiworanan et al., 2017)

Table 1. Location and characteristics of sampling points (1–5) of the studied hot springs

Spring (Site)	Location Co–ordinates	Point 1			Point 2			Point 3			Point 4			Point 5		
		Disturbance characteristics	Altitude (m.a.s.l)	Substrate	Disturbance characteristics	Altitude (m.a.s.l)	Substrate	Disturbance characteristics	Altitude (m.a.s.l)	Substrate	Disturbance characteristics	Altitude (m.a.s.l)	Substrate	Disturbance characteristics	Altitude (m.a.s.l)	Substrate
SK (Hot stream)	18° 48' 59" N 99° 13' 39" E	HTA	360	gravels, sand, soil sediment	HTA	360	gravels, sand, soil sediment	HTA	360	Concrete	LTA	360	Concrete	NTA	360	Concrete
TPN (Hot stream)	18° 16' 16" N 98° 23' 42" E	NTA	372	forest, cobbles	NTA	320	gravels, sand	NTA	363	sand, soil sediment	NTA	369	sand, soil sediment	NTA	351	Concrete
TP (Hot stream)	19° 17' 53" N 98° 28' 14" E	LTA	518	forest, gravels, sand	NTA	536	cobbles, gravels	NTA	505	sand, soil sediment	NTA	481	sand, soil sediment	NTA	524	sand, soil sediment
CHD (Hot pool)	19° 35' 49" N 98° 56' 43" E	LTA	485	rocks, sand	LTA	491	rocks, sand	LTA	497	rocks, sand	–	–	–	–	–	–
JS (Hot stream)	18° 50' 12" N 99° 28' 19" E	HTA	424	gravels, sand, small rocks	HTA	424	gravels, sand, small rocks	HTA	424	gravels, sand, small rocks	HTA	424	gravels, sand, small rocks	HTA	430	gravels, sand, small rocks
MJ (Hot pool)	17° 58' 44" N 99° 38' 15" E	HTA	143	rock, soil sediment	HTA	143	Hot pool, rock, soil sediment	HTA	143	rock, soil sediment	NTA	131	Concrete	–	–	–
SL (Hot pool and cold stream)	18° 05' 35" N 99° 49' 45" E	NTA	131	gravels, sand	–	–	–	–	–	–	–	–	–	–	–	–
NG (Hot pool, forest and cold stream)	19° 13' 19" N 100° 39' 12" E	NTA	408	gravels, rocks, sand	NTA	408	gravels, rocks, sand	NTA	408	gravels, rocks, sand	–	–	–	–	–	–

The abbreviations of springs names are provided in Fig. 1.
Abbreviations: HTA – High tourist attraction, NTA – None tourist attraction, LTA – Low tourist attraction.

Table 2. Range of physical and chemical characteristics obtained at the sampling points (n = 3) in the eight hot spring sampling sites (springs abbreviations in Table 1)

Spring and Site	pH	Cond. (µS cm^{-1})	NO_3^- (mg L^{-1})	NH_4^+ (mg L^{-1})	SRP (mg L^{-1})	SiO_2 (mg L^{-1})	S_2 (mg L^{-1})	HDN (mg L^{-1} $CaCO_3$)	Alk. mg.l^{-1} $CaCO_3$	Water temp. (°C)
SK1	8.81–8.89	759–775	0.5–0.8	0.66–0.77	0.3–0.84	117.6–143	0.085–0.3	8–8	339–347	37–39.4
SK2	8.72–8.81	813–828	0.4–0.5	0.55–0.75	0.36–0.67	128.4–132	0.013–0.02	4–4	333–338	41.2–44.8
SK3	8.73–8.82	771–790	0.4–0.4	0.5–0.55	0.65–1.21	125.8–137	0.01–0.013	4–12	346–352	45.8–49.2
SK4	8.74–8.78	798–829	0.7–1.7	1.09–1.24	0.67–0.97	145–154	0.302–1.094	4–8	331–349	51.6–54.4
TPN1	8.15–8.53	554–572	0.2–0.3	0.15–0.19	1.07–1.28	72.8–120	0.001–0.006	12–16	229–229	38.1–40
TPN2	8.46–8.57	543–561	0.2–0.4	0.16–0.23	0.94–1.56	114.2–117	0.001–0.002	12–16	220–224	41.1–43.1
TPN3	8.47–8.63	543–560	0.3–0.5	0.13–0.26	0.89–1.3	110.6–118	0–0	8–12	223–226	45.2–45.2
TPN4	8.32–8.50	571–580	0.3–0.3	0.16–0.2	1.1–1.29	108–118	0.002–0.004	8–16	219–222	54.5–55.3
TPN5	8.09–8.43	503–537	0.6–1.1	0.3–0.41	1.07–1.59	104–105	0.043–0.071	12–16	215–219	85–85
TP1	6.96–7.14	373–384	0.2–0.5	0.01–0.03	0.04–0.13	71–72.2	0–0	36–52	156–184	37.2–39.8
TP2	7.16–7.32	360–384	0.2–0.3	0.03–0.06	0.07–0.12	70–74.8	0–0	36–44	154–158	41.9–44.5
TP3	7.29–7.40	372–382	0–0.4	0.01–0.04	0.01–0.18	69.4–71.3	0–0	28–40	154–168	37.2–49.3
TP4	6.88–7.31	369–381	0–0.3	0.03–0.09	0.19–0.37	68–69.2	0–0.01	36–48	154–168	50.8–54.4
CHD1	7.13–7.32	803–807	0.1–0.3	0.37–0.39	0.86–1.09	55.1–55.9	0–0.004	52–64	443–449	37.8–39.9
CHD2	6.97–7.16	791–809	0.3–0.4	0.36–0.37	0.85–1.13	54.1–55.6	0.001–0.002	52–60	437–441	42.2–42.9
CHD3	7.17–7.29	795–801	0.2–0.4	0.4–0.44	0.94–0.55	55.1–58.8	0–0.001	52–64	434–438	45.4–45.5
JS1	7.36–7.74	563–580	0.1–0.2	0.23–0.25	0.41–0.53	94.2–96.8	0.002–0.004	40–52	230–235	39.8–40.2
JS2	7.55–7.63	558–573	0.2–0.3	0.34–0.37	0.18–0.19	94.2–96.9	0.002–0.005	60–72	238–285	41.6–44.5
JS3	7.61–7.74	548–568	0.1–0.2	0.32–0.34	0.19–0.23	92.8–97	0.002–0.004	52–60	236–240	47.1–47.3
JS4	7.55–7.62	521–530	0.2–0.4	0.35–0.38	0.1–0.74	92.1–95.2	0.003–0.007	52–68	218–232	51.2–54.4
MJ2	7.31–7.48	1022–1135	0.4–0.8	0–0.27	0.1–0.62	83.3–85.9	0–0.001	64–64	360–368	41.1–41.2
MJ3	7.37–7.43	1012–1016	0.2–0.3	0–0	0.32–0.9	83.1–83.9	0–0	64–72	350–356	47.9–50.1
MJ4	7.34–7.41	1009–1019	0.1–0.6	0–0.15	0.29–0.55	81.9–82.3	0–0.001	64–76	352–354	54.1–54.1
SL1	7.50–7.69	1066–1126	0.4–0.5	0.18–0.28	0.24–1.07	82.3–83.3	0.002–0.004	48–53	414–502	41.3–42.3
NG1	7.04–7.27	485–562	0.2–0.4	0–0.1	0.22–0.34	34.2–36.4	0.001–0.002	48–64	130–178	38.3–39.7
NG2	7.01–7.08	622–652	0.2–0.3	0–0.08	0.27–0.54	38.5–39.7	0–0.003	40–40	182–192	42.4–43.9
NG3	7.07–7.23	665–692	0.3–0.4	0.01–0.21	0.49	23.9–41.9	0.001–0.002	24–32	194–202	46.2–47.6

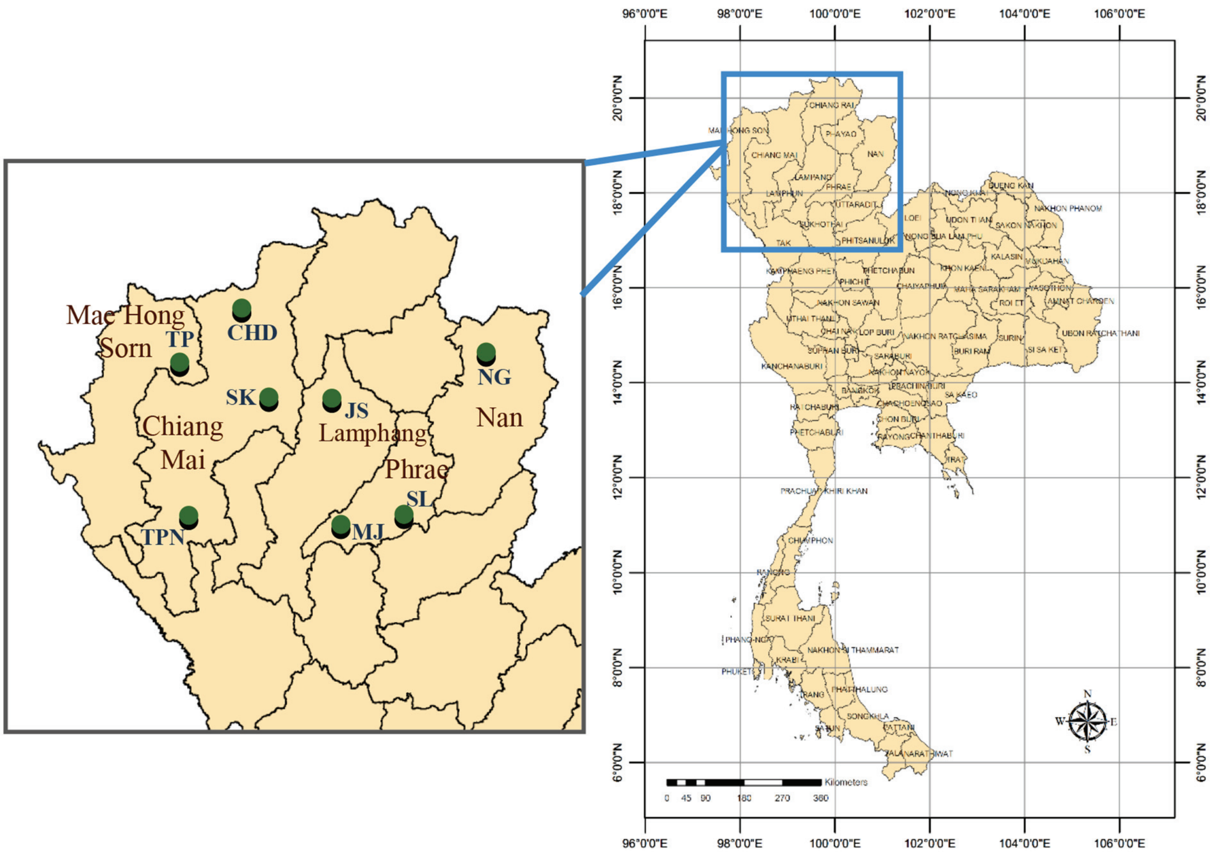

Fig. 2. Map of Thailand showing eight sampling sites of hot springs in northern Thailand: SK – San Kamphaeng hot spring, Chiang Mai province; TPN – Theppanom hot spring, Chiang Mai province; TP – Ta Pai hot spring, Mae Hong Son province; CHD – Pong Ang hot spring, Chiang Mai province; JS – Chae Sorn hot spring, Lampang province; MJ – Mae Chok hot spring, Phrae province; SL – Wat Salaeng hot spring, Phrae province; NG – Pong Gi hot spring, Nan province

Statistical Computing version 3.4.2 supported by CRAN (R Core Team, 2017). Sampling sites were grouped based on water properties using Hierarchical Cluster Analyses (HCA) and carried out with h cluster function of R using Euclidean distance. The complete option was embraced for the clustering as it includes a greater proportion of the information than other options. A non-metric multidimensional scaling technique (NMDS) with Bray–Curtis distance measure was used to evaluate variations in diatom assemblages and the water quality of each sampling site in the studied hot springs. The physico-chemical factors were correlated to the NMDS axes using the Fits an Environmental Vector (envfit function) of the library (vegan) (Oksanen et al., 2018). Environmental variables were transformed using square-root transformation and standardized by Wisconsin command. The fit (R^2) of each variable to the ordination was assessed using the envfit function with Monte-Carlo analysis of 999 permutations. The NMDS result was plotted using ggplot function of the gglpot2 library (Wickham, 2009).

RESULTS AND DISCUSSION

Diversity of hot spring diatoms and relationship of benthic diatoms to chemical and physical properties

A total of 46 species of hot spring diatoms were identified in this study (Figs 3–6), predominantly pennates. The list of the determined diatoms with their relative abundance is shown in Table 3. The dominant genera according to high relative abundance (more than 1%) were *Diatomella* (41.7%) followed by *Achnanthidium* (20.9%), *Anomoeoneis* (11.2%), *Rhopalodia* (6.4%), *Sellaphora* (5.7%), *Navicula* (2.9%), *Nitzschia* (2.4%) and *Craticula* (2.1%).

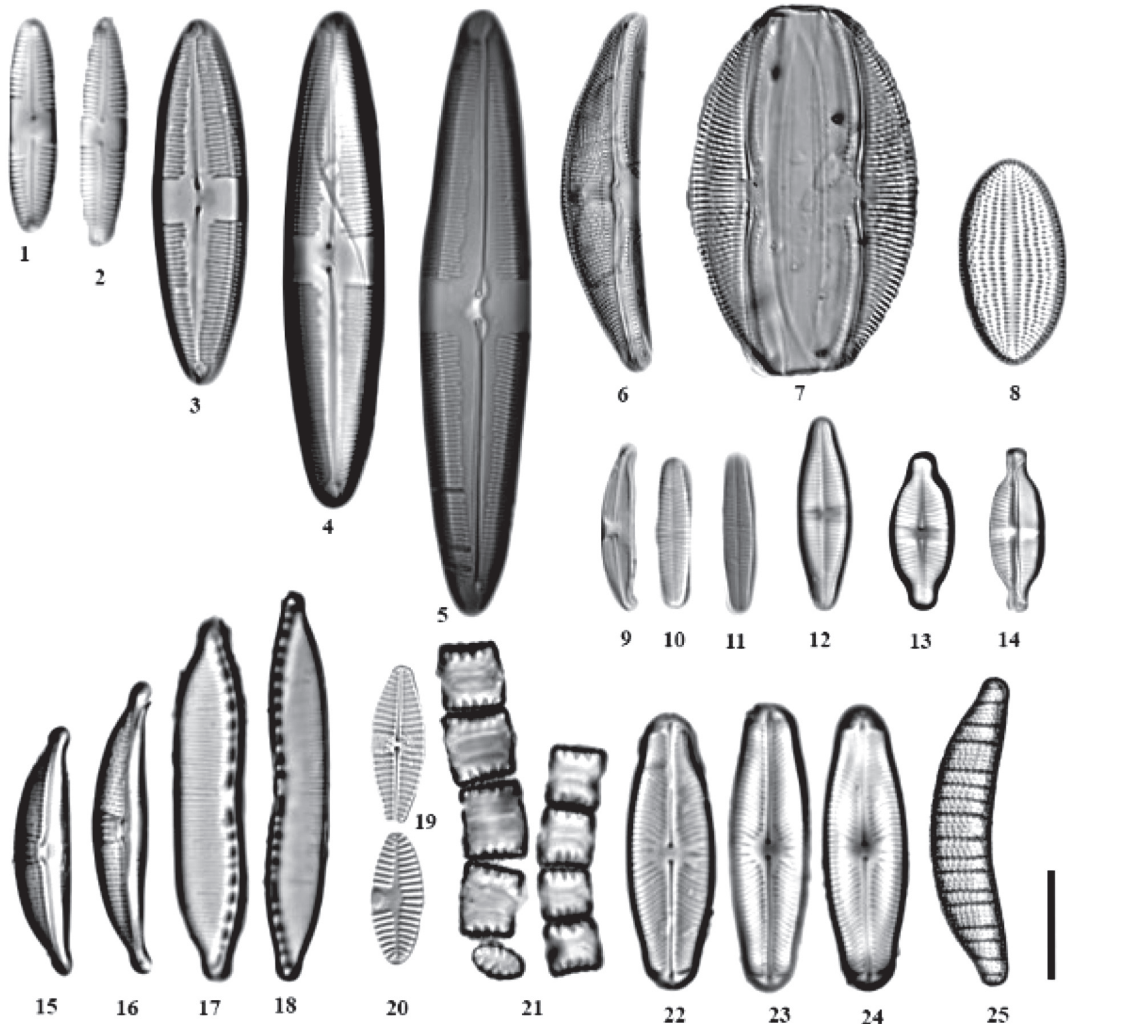

Fig. 3. Light micrographs (LM) of sampled material: (1–2) *Caloneis bacillum*, (3–5) *C. molaris*, (6–7) *Amphora ovalis*, (8) *Cocconeis placentula*, (9) *Halamphora montana*, (10–11) *Achnanthidium minutissimum*, (12) *Achnanthidium* sp., (13–14) *Achnanthidium exiguum*, (15–16) *Halamphora fontinalis*, (17–18) *Hantzchia amphioxys*, (19–20) *Planothidium lanceolatum*, (21) *Pseudostaurosira elliptica*, (22–24) *Sellaphora lanceolata,* (25) *Epithemia adnata* (= syn. *E. zebra* (Ehrenberg) Kützing 1844). Scale bar = 10 µm

These genera included 16 species, i.e. *Achnanthidium exiguum*, *Anomoeoneis sphaerophora*, *Anomoeneis* sp$_3$, *Craticula acidoclinata*, *Craticula ambigua*, *Craticula cuspidata*, *Diatomella balfouriana*, *Navicula grimmei*, *Navicula rostellata*, *Navicula subrhynchocephala*, *Nitzschia amphibia* Grunow, *Nitzschia clausii*, *Nitzschia ignorata*, *Nitzschia palea*, *Rhopalodia gibberula* and *Sellaphora lanceolata*.

A. exiguum, *D. balfouriana* and *A. sphaerophora* were the dominant species at almost all sampling sites, and this finding was similar to that reported by Owen & Renaut (2008), who have stated that the most common taxa, including *A. exiguum* var. *heterovalvum*, *A. sphaerophora*, occur in Kenyan springs. Also, *A. exiguum*, *G. parvulum*, *A. ovalis* have been previously reported as thermophiles existing at 40–58°C (Quintela et al., 2013; Covarrubias et al., 2016). Moreover, *A. exiguum* has been recorded in all samples collected from the limnocrene springs of Bunica that are situated in the south of Bosnia and Herzegovina, and this species has been frequently found in many alkaline hot springs (Dedić et al., 2015).

Furthermore, *Caloneis molaris* (Fig. 3 (3–5)), *Craticula acidoclinata* (Fig. 5 (60)), *Pinnularia saprophila* (Fig. 6 (63–64)) and *Navicula subrhyn-*

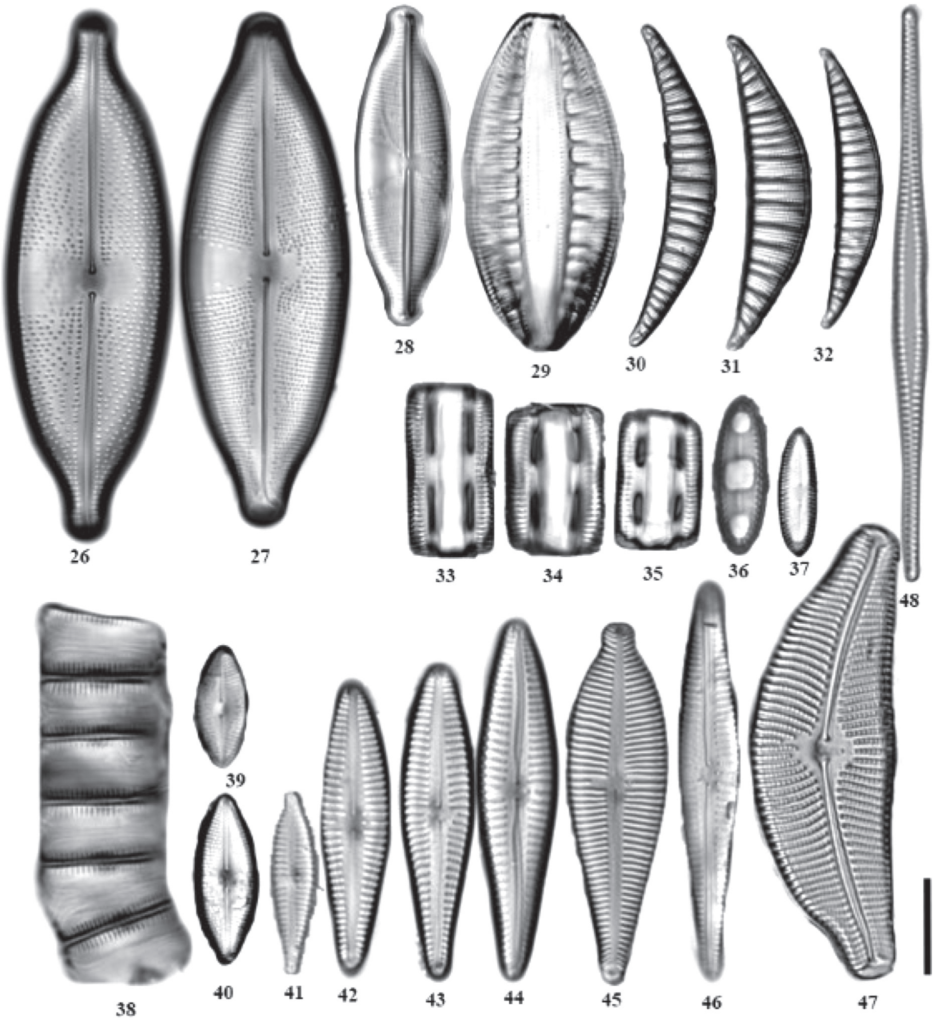

Fig. 4. Light micrographs (LM) of sampled material: (26–27) *Anomoeoneis sphaerophora*, (28) *Anomoeneis* sp., (29–32) *Rhopalodia gibberula*, (33–37) *Diatomella balfouriana*, (38–40) *Diadesmis confervacea*, (41) *Gomphonema parvulum*, (42–44) *Gomphonema affine*, (45) *Gomphonema sphaerophorum*, (46) *Gomphonema gracile*, (47) *Cymbella tumida*, (48) *Fragillaria crotonensis*. Scale bar = 10 µm

*chocephal*a (Fig. 6 (77)) have been determined to be newly recorded species in Thailand. They were compared to the freshwater algae checklist published in Thailand along with other related reference materials (Lewmanomont et al., 1995; Pekthong, 2002; Pekthong & Peerapornpisal, 2001; Kunpradid, 2005; Suphan, 2004, 2009; Inthasotti, 2006a, b; Leelahakriengkrai, 2007, 2011; Pruetiworanan, 2008; Yana 2010, 2014; Suphan & Peerapornpisal, 2010; Nakkaew, 2015). The recorded species of hot spring diatoms new to Thailand in this study were found in fewer numbers than those recorded in earlier reports published in Thailand. This ocurred because we collected samples from specific environments, where microbes were only able to survive under harsh conditions such as high temperature, pH and alkalinity. These factors restricted the type and number of diatom species that were present.

Results from the statistical tests indicated a higher taxonomic richness at SK sampling site compared to the other sampling sites. The results of Shannon's diversity index ranged from 0.000 to 2.365 and the evenness ranged from 0.000 to 0.875, whereas the species richness was found to range from 0 to 24. The lowest values of the diversity index and the evenness values were observed at sampling sites TPN5, TP4, JS4 and MJ4, whereas the highest values were observed at sampling site SK1. The highest values of evenness were revealed at sampling site CHD1. The highest numbers of species were recorded at sampling site SK1, while the lowest values were recorded at sampling sites TPN5, TP4, JS4 and MJ4. The number of species at most sampling sites was found to have decreased as the water temperature was higher.

The NMDS ordination revealed differences in species composition in eight different springs (Fig. 8). The data stress from NMDS analysis was 0.17, which indicated an acceptable ordination summarizing the observed distances among the samples (Kruskal & Wish, 1978; Legendre & Legendre, 1998). Subsequent to the environmental variables, ten variables were included in the analysis and fitted to the ordination space: pH, conductivity, NO_3^-, NH_4^+, SRP, SiO_2, S^{2-}, total hardness, alkalinity and water temperature. Based on R^2 values, silicon dioxide (SiO_2), pH, conductivity, water temperature and total hardness had a strong relationship with species composition, respectively (Table 4).

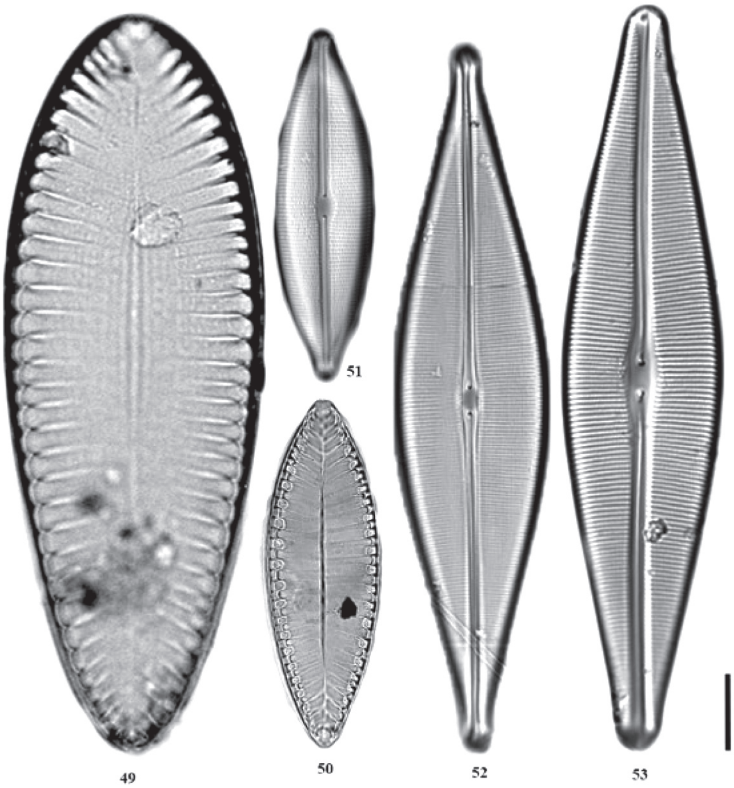

Fig. 5. Light micrographs (LM) of sampled material: (49) *Surirella elegans*, (50) *Surirella amphioxys*, (51) *Craticula ambigua*, (52) *Craticula cuspidata,* (53) *Craticula acidoclinata*. Scale bar = 10 µm

The diatom taxa in the NMDS ordination diagram indicated that the increase of silicon showed a positive correlation to the diversity of diatoms. Silicon oxide is thus essential to diatoms, as an insufficient supply of Si in the environment will prevent diatoms from flourishing or can result in teratological specimens (Seckbach, 2007).

However, silicon concentration does not appear to be the only limiting factor in the distribution of diatoms. Our data specify that pH and silicon concentrations seem to be an imperative factor in hot spring diatom distribution, in the same way they do in many alkaline springs found elsewhere in continental Europe (Cantonati & Lange-Bertalot, 2010; Kollár et al., 2015; Mogna et al., 2015) and Majorca island (Delgado et al., 2013). The species associated with higher pH and SiO_2 values in this study were *Caloneis aequatorialis*, *Cocconeis placentula*, *Craticula cuspidata*, *Diploneis elliptica*, *Gomphonema affine*, *Gomphonema augur*, *Halamphora fontinalis*, *Planothidium lanceolatum* and *Stauroneis anceps*. These conditions were relevant to the SK1 and SK2 samples. Consistent with Leira et al. (2017), who have found *Planothidium lanceolatum* in water bodies of slightly alkaline pH conditions in thermo-mineral springs of Galicia (NW Spain).

Another factor that affected diatom diversity was conductivity. In this study, *Achnanthidium exiguum*, *Sellaphora lanceolata* and *Pinnularia abaujensis* had positive relationship with conductivity that occurred in the MJ2 and MJ3 samples. Mangadze et al. (2017) have revealed that diatom assemblages are proper indicators of ionic composition/conductivity and stream size in lotic ecosystems. Also, it is well known that conductivity and ionic composition have impact on diatom distribution (Bere & Tundisi, 2011). Conductivity is highly correlated with variables that directly affect diatom assemblage composition and individual species responses along the gradient (Ryves et al., 2002).

Water temperature showed negative influence on

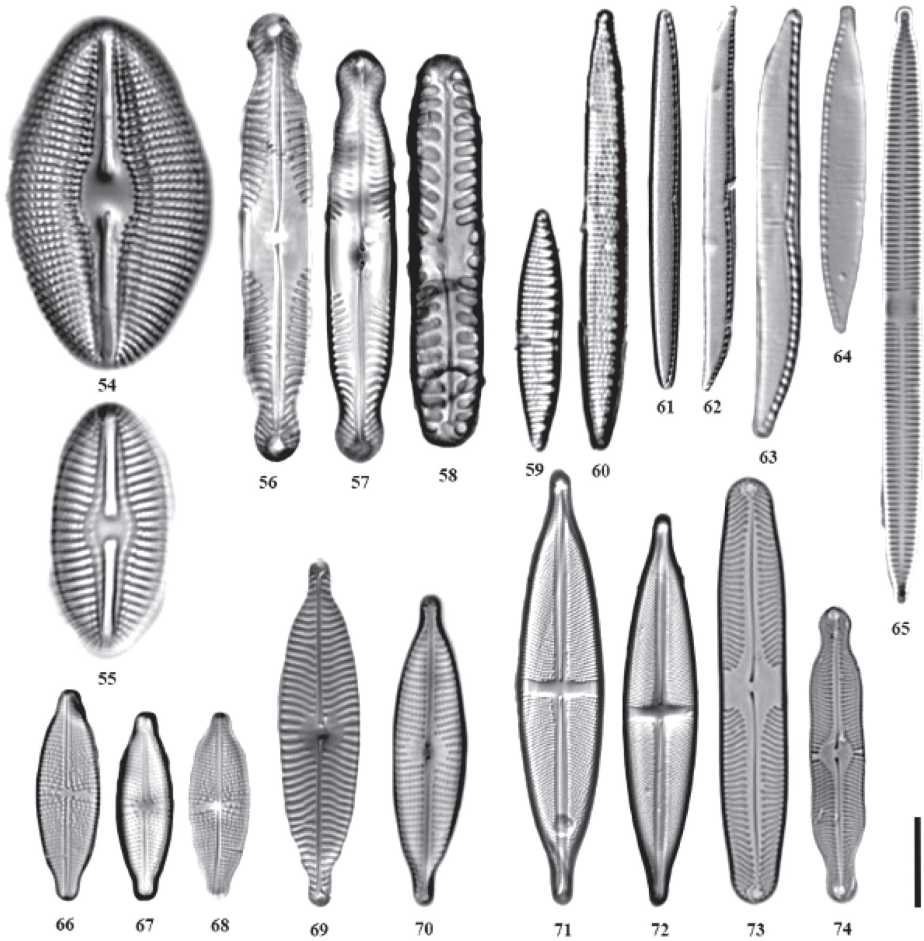

Fig. 6. Light micrographs (LM) of sampled material: (54) *Diploneis elliptica*, (55) *Diploneis subovalis*, (56–57) *Pinnularia saprophila*, (58) *Pinnularia borealis*, (59–60) *Nitzschia amphibia*, (61) *Nitzschia ignorata*, (62–63) *Nitzschia clausii*, (64) *Nitzschia palea*, (65) *Synedra ulna*, (66–68) *Navicula kotschyi*, (69) *Navicula rostellata*, (70) *Navicula subrhynchocephal*a, (71–72) *Stauroneis anceps*, (73) *Pinnularia abaujensis*, (74) *Pinnularia interrupta*. Scale bar = 10 μm

the diversity of diatoms. Only *Amphora montana* was allocated near the temperature arrow, which indicated that this species was more abundant at the higher temperature sampling sites. This finding was in accordance with the findings of NIKULINA & KOCIOLEK (2011), who have observed that the diversity of diatom taxa present in hot springs decrease significantly when water temperature exceeds 70°C. These findings are also in agreement with MOGNA et al. (2015). However, the relative abundance of some diatom change in hot springs was not consistent with the temperature, which means that other climatic variables were relevant.

Total hardness positively corelated with *Amphora ovalis*, *Craticula acidoclinata*, *Craticula ambigua*, *Navicula grimmei*, *Surirella elegans* (Fig. 8). The species occurred at the sites with high conductivity and total hardness. These sites were situated in a mountainous limestone area and were affected by the presence of carbonate and bicarbonate ions from limestone. Naturally, limestone areas contain three carbonate types ($H_2CO_3^-$, $HCO_3^-$and CO_3^{2-}) that can affect the alkalinity of a water body in a given area. If waters flow through limestone regions or bedrock areas that contain carbonates, they tend to have high alkalinity values, which can lead to high conductivity levels (CRAVOTTA, 2003). Similar results have been obtained by other authors, e.g. GESIERICH & KOFLER (2010), who have studied springs situated in the central Alps in Austria and have found that conductivity and nitrates are the most relevant differentiating variables of diatom assemblage composition.

Table 3. Species composition and relative abundance of diatoms at the sampling points

Taxon name	Abbreviations	SK	TPN	TP	CHD	JS	MJ	SL	NG
Achnanthidium minutissimum (Kützing) Czarnecki	Achmin	0–1	0	0–1	0–3	0–2	0	0	0
Achnanthidium exiguum (Grunow) Czarnecki	Achexi	0–2	0–3	0–3	0–5	0	0–4	0	0–5
Amphora montana Krasske	Ampmon	0–2	0–1	0–1	0–2	0–1	0	0	0
Amphora ovalis (Kützing) Kützing	Ampova	0–1	0–1	0	0–1	0	0	0	0
Anomoeoneis sphaerophora (Ehrenberg) Pfitzer	Anopha	0–2	0–1	0	0	0–1	0	4–5	0–3
Anomoeneis sp.3	Anosp3	0–1	0	0	0	0	0	0–1	0
Caloneis aequatorialis Hustedt	Calaeq	0–1	0	0	0	0	0	0	0
Caloneis bacillum (Grunov) Mereschkowsky	Calbac	0–1	0	0	0	0	0	0	0
Cocconeis placentula Ehrenberg	Cocpla	0–1	0–1	0	0	0	0	0	0
Craticula acidoclinata Lange-Bertalot & Metzeltin	Craaci	0–2	0	0	0–3	0	0	0	0
Craticula ambigua (Ehrenberg) Mann in Round, Crawford & Mann	Craamb	0	0	0	0–1	0	0	0	0
Craticula cuspidate (Kützing) Man	Cracus	0–2	0–1	0	0	0–2	0	0–1	0
Cymbellatumida (Brébisson) Van Heurck	Cymtum	0–1	0	0	0–1	0–1	0	0–1	0–2
Diadesmis confervacea Kützing	Diacon	0	0	0	0	0	0	0–1	0
Diatomella balfouriana Greville	Diabal	1–5	1–5	2–5	0–5	3–5	0	0	0–4
Diploneis elliptica (Kützing) Cleve	Dipell	0–1	0	0	0	0	0	0	0
Diploneis ovalis (Hilse) Cleve	Dipova	0–1	0–1	0–1	0	0	0	0	0
Epithemia zebra (Ehrenberg) Kützing	Epizeb	0–1	0	0	0	0	0–3	0	0
Fragillaria crotonensis Kitton	Fracro	0–1	0	0	0	0	0–1	0	0
Gomphonema affine Kützing	Gomaff	0–1	0–1	0–1	0	0–1	0	0	0
Gomphonema augur Ehrenberg	Gomaug	0–1	0	0	0	0–1	0	0	0
Gomphonema gracile Ehrenberg	Gomgra	0–1	0	0	0	0	0	0	0
Gomphonema parvulum (Kützing) Van Heurck	Gompar	0–1	0	0	0	0–1	0	0	0
Halamphora fontinalis (Hustedt) Z. Levkov	Halfon	0–1	0	0	0	0	0	0	0
Hantzchia amphioxys (Ehrenberg) Grunow in Cleve et Grunow	Hanamp	0–1	0–1	0	0	0–1	0	0	0
*Navicula grimmei*Krasske in Hustedt	Navgri	0	0	0–1	0–5	0	0–2	0–1	0–4
Navicula rostellata (Kützing) Cleve	Navros	0	0	0–1	0	0	0	0	0
*Navicula subrhynchocephal*a Hustedt	Navsub	0	0	0	0	0	0	0	0–1
Nitzschia amphibian Grunow	Nitamp	0	0–1	0	0	0	0	0	0
Nitzschia clausii Hantzsch	Nitcla	0–1	0	0	0	0	0–2	0	0
Nitzschia ignorata Krasske	Nitign	0–1	0	0	0	0	0	0	0
Nitzschia palea (Kützing) W. Smith	Nitpal	0–1	0–1	0–1	0–2	0–1	0–4	0	0
Pinnularia abaujensis (Pantoscek) Ross	Pinaba	0–1	0	0	0	0–1	0–1	0	0
Pinnularia capitata Ehrenberg	Pincap	0	0–1	0–1	0	0	0	0	0
Pinnularia lapponica Hustedt	Pinlap	0	0	0–1	0	0	0	0	0
Pinnularia mesolepta (Ehrenberg) Smith	Pinmes	0	0	0	0	0–1	0	0–1	0
Planothidium lanceolatum (Breb.) Round &Bukhtiyarova	Plalan	0–1	0–1	0–1	0	0	0	0	0
Rhopalodia gibberula (Ehrenberg) O.F. Müller	Rhogib	0–3	0–3	0–1	0–5	0–1	0–5	0	0–1
Sellaphora lanceolata D.G. Mann & S. Dropp in Mann et al.	Sellan	0–1	0–2	0–2	0–5	0–1	0–5	1	0–5
Stauroneis anceps Ehrenberg	Staanc	0–1	0–1	0	0	0	0	0	0

Taxon name	Abbreviations	SK	TPN	TP	CHD	JS	MJ	SL	NG
Staurosira elliptica(Schumann) D.M.Williams & Round	Staell	0	0–1	0	0	0	0	0	0
Surirella biseriata Brébison	Surbis	0–1	0–1	0	0	0	0	0	0
Surirella elegans Ehrenberg	Surele	0	0	0–1	0–1	0	0–1	0–1	0–1
Synedra ulna (Nitzsch) Ehrenberg	Synuln	0–1	0	0	0–1	0	0	0	0

Note: Relative abundance of diatoms: 0 = absence, 1 = (0–20)% of relative abundance, 2 = (21–40), 3 = (41–60), 4 = (61–80), 5 = (81–100).
SK – San Kamphaeng hot spring, Chiang Mai province; TPN – Theppanom hot spring, Chiang Mai province; TP – Ta Pai hot spring, Mae Hong Son province; CHD – Pong Ang hot spring, Chiang Mai province; JS – Chae Sorn hot spring, Lampang province; MJ – Mae Chok hot spring, Phrae province; SL – Wat Salaeng hot spring, Phrae province; NG – Pong Gi hot spring, Nan province.

From the NMDS analysis, *Diatomella balfouriana,* which could be found in various hot springs, showed a non-relationship of the water properties. Sompong (2001) has reported that *D. balfouriana* is a dominant species at temperatures between 30 and 59°C, whereas only a small number of the publications in the world have identified this species as a dominant species in hot springs.

The results of this study seem to indicate that it is difficult to establish a characteristic diatom flora for hot spring waters. In fact, none of the taxa were common to eight hot springs. In any case, there are different species for which the physical and chemical characteristics of the individual hot spring conditions might be more conclusive in the controlling appropriation.

Hot springs offer the opportunity to test hypotheses regarding the biogeography of microbial organisms. Kociolek & Spaulding (2000) have detailed some families, genera, and species that appear to be localized with regard to their distribution. However, the situation that different habitats can be located in very different geographic locales but still may have similar temperatures and water chemistries could be informative of hot spring ecosystems in general. This has led to the study of the factors that support similar or different species of diatoms (Vyverman et al., 2007). Likewise, Owen & Renaut (2008) have compared hot springs in Iceland, New Zealand and Kenya, and noted that the dominant taxa that are present in these systems are all quite notable regarding the significant groups represented. With regard to certain diatoms found in South Africa (*Synedra*, *Aulacoseira*, *Nitzschia*, *Cyclotella*, *Gyrosigma*, *Craticula*), these occurred exclusively at temperatures of < 45°C and pH values of < 8 (Jonker et al., 2013).

Additionally, sediments can be seen as living spaces for benthic organisms, where they offer a place for resting and refugia. Organisms can be enlisted or re-suspended from sediments in the water body (Dobson & Frid, 2008). Moreover, residue provides a source of chemical compounds that exist in the catchment area. All the hot spring sampling sites were approachable to the public, while some were particularly attractive as tourist destinations and were serviced as catering resources. Thus, human activities may have led to disturbance or disruption of the fluctuations of physicochemical features and diatom distribution at some of these hot springs. The sampling intention did not recognize the scope to which human activities had changed the natural ecosystem and impacted upon the diversity of the diatom communities. Thus, to evaluate the effects of human disturbances, continuous monitoring should be done.

Physicochemical properties of water collected from eight hot springs

Previous surveys have indicated that the surface temperatures of hot springs in northern Thailand are between 40–100°C. The range of water temperatures in this study was between 38.4 and 85°C (Table 2). This is attributable to the mixing of geothermal water with groundwater during the course of circulation and at the time the water flows to the surface (Raksaskulwong, 2002). Moreover, most of the hot springs in Thailand are known to be alkaline hot springs and are found in territories, where the pH value is over seven and can be as high as 11 or 12. During the course of this research, the range of pH values was from 6.8 to 8.8, which correlated with the expected hot spring conditions for this area. Additionally, hot springs in this region are typically discovered close to the areas with a lot of limestone or dolostone (Kruse, 1997), where a broad measure of silica material can serve as a host for algal mats. Nutrient levels (nitrate, ammonia, phosphate) are relatively low, except at SK4, which is the main point of attraction for tourists. The main touristic activity in the hot springs of Thailand involves the boiling of "Onsen" eggs, which may result in high levels of nutrient contamination to the environment.

HCA was used to analyse 31 sampling points from eight hot springs. Cluster analyses for the whole dataset produced similar water quality patterns. Clustering depends on the place that cutoff lines are drawn. The best number of clusters was determined using the Kmean method by NbClust function of the NbClust library in the R programming environment. It was found that the sampling points were divided into three groups and the similarity of their water properties was highlighted on the heat map (Fig. 7). Overall, the sampling points were clustered according to the hot spring sites. Group 1 was the largest group including the sampling points located at SL, MJ, CHD, NG, TP and JS. These sampling points were clustered according to a positive correlation with conductivity, alkalinity and total hardness. Group 2 included the sampling points from TPN and SK1-3. These sampling points were separated from group 1 with a positive correlation with pH, SiO_2 content and soluble reactive phosphorus levels. Group 3 contained only

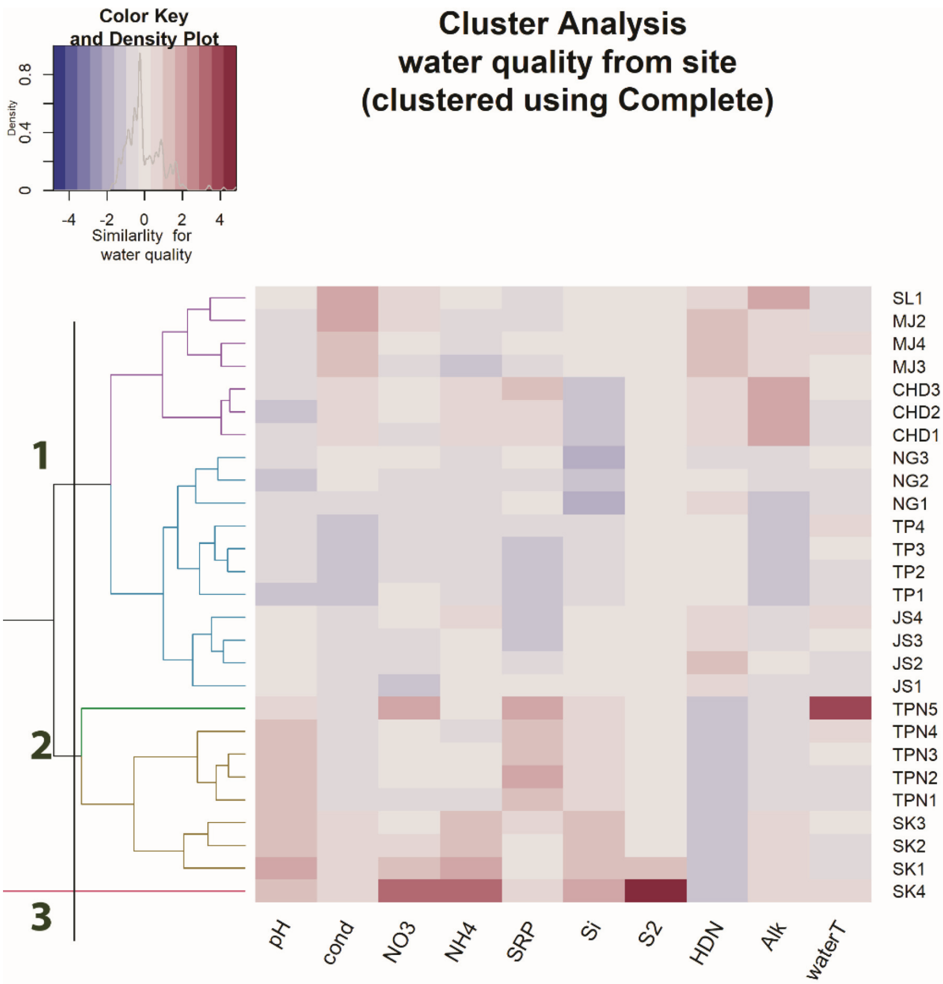

Fig. 7. Cluster dendrogram and heat map of sampling sites according to the physicochemical factors in eight hot spring sampling points analysed by the Complete method

Table 4. Relationships between the species ordination scores (NMDS) and the influenced environmental factors

	NMDS1	NMDS2	R^2	p
pH	0.20429	-0.97891	0.3840	0.002 **
Cond. (µS cm^{-1})	-0.94098	0.33847	0.3720	0.007**
NO_3^- (mg L^{-1})	0.60151	-0.79887	0.0285	0.699
NH_4^+ (mg L^{-1})	0.61863	-0.78568	0.1907	0.084
SRP (mg L^{-1})	0.21071	0.97755	0.0099	0.895
SiO_2 (mg L^{-1})	0.30347	-0.95284	0.4242	0.001***
S_2 (mg L^{-1})	0.90644	-0.42234	0.0556	0.507
HDN (mg L^{-1} $CaCO_3$)	-0.24303	0.97002	0.3426	0.012*
Alk. (mg.L^{-1} $CaCO_3$)	-0.86020	0.50995	0.1614	0.121
Water Temp. (°C)	0.87696	0.48056	0.3590	0.001***

Significant codes: (***): $p < 0.001$, (**): $p < 0.01$, (*): $p < 0.05$; Permutation: free, number of permutations – 999.

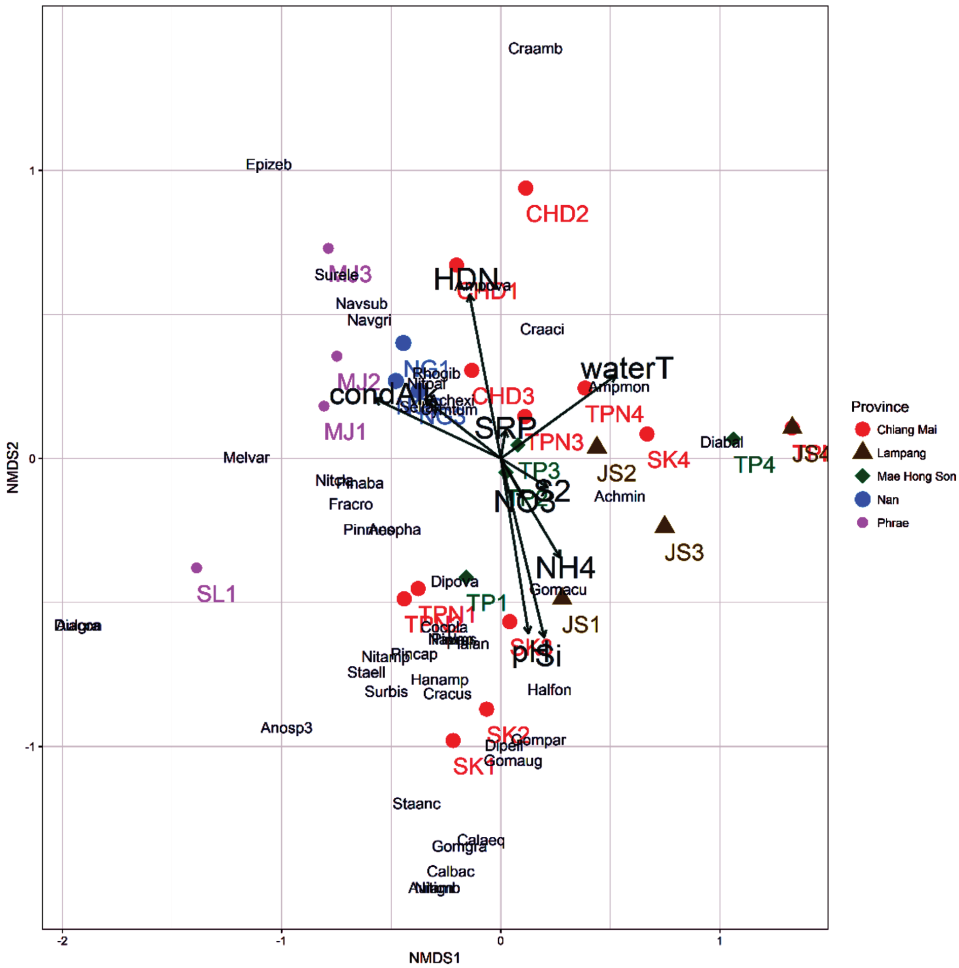

Fig. 8. Non-metric multidimensional scaling (NMDS) ordination diagram of the hot springs based on species composition. Ten environmental factors were evaluated and indicated by green arrows: HDN – total hardness, SRP – soluble reactive phosphorus, waterT – water temperature, cond – conductivity, Si – SiO_2, Alk – alkalinity. Orientation and length indicate direction of the change and strength of correlation, respectively. Correlation data are shown in Table 4.

one sampling point from SK4. It was distinguished from the other sampling points because of the high nutrient levels and high sulphur concentrations that were present.

CONCLUSIONS

Corresponding to our preliminary results, it should be concluded that the community diversity of hot spring diatoms at eight hot spring sampling sites was established by classification of forty six species. These belonged to 2 classes, 14 orders, 18 families and 27 genera. Of these, four species, *Caloneis molaris*, *Craticula acidoclinata*, *Navicula subrhynchocephala* and *Pinnularia saprophila*, were determined to be records new to Thailand. The dominant genera according to high relative abundance (more than 1%) were *Diatomella* (41.7%) followed by *Achnanthidium* (20.9%), *Anomoeoneis* (11.2%), *Rhopalodia* (6.4%), *Sellaphora* (5.7%), *Navicula* (2.9%), *Nitzschia* (2.4%) and *Craticula* (2.1%). The first three dominant species were *Diatomella balfouriana*, *Achnanthidium exiguum* and *Anomoeoneis sphaerophora*. Nonetheless, there are some species that could grow within a wide range of water properties such as *A. exiguum*. Insignificant differences were found in terms of species richness or the diversity index values, but silicon dioxide (SiO_2), pH, conductivity, water temperature and total hardness were the main environmental factors that influenced certain specific diatom assemblages.

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ŠIAURĖS TAILANDO KARŠTŲ ŠALTINIŲ TITNAGDUMBLIAI

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Santrauka

Titnagdumblių įvairovė buvo tirta Šiaurės Tailando karštuosiuose šaltiniuose. Keturiasdešimt šešios rūšys buvo rastos aštuoniose tyrimų vietovėse, iš jų pagal santykinį gausumą dominavo *Diatomella balfouriana* (41.7%), *Achnanthidium exiguum* (20.9%) ir *Anomoeoneis sphaerophora* (11.2%). Tuo tarpu *Caloneis molaris*, *Craticula acidoclinata*, *Navicula subrhynchocephala* ir *Pinnularia saprophila* buvo pirmą kartą identifikuotos Tailando vandens telkiniuose. Statistinė analizė, naudojant neparametrinį daugiamačių skalių metodą, parodė titnagdumblių floros skirtumus aštuoniuose karštuosiuose šaltiniuose ir jų sąryšį su skirtingais aplinkos veiksniais. Silicio dioksidas (SiO_2), pH, vandens savitasis elektrinis laidis, vandens temperatūra ir kietumas buvo statistiškai reikšmingi faktoriai *Achnanthidium exiguum*, *Amphora montana*, *Caloneis aequatorialis*, *Cocconeis placentula*, *Craticula cuspidata*, *Diploneis elliptica*, *Gomphonema affine*, *Gomphonema augur*, *Halamphora fontinalis*, *Planothidium lanceolatum*, *Pinnularia abaujensis*, *Sellaphora lanceolata* ir *Stauroneis anceps* rūšių gausumui.