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Report

Appearance of *Euglena mutabilis* with a filamentous green alga *Klebsormidium* community near a volcanic acidified spring in the southern part of Mt. Ontake, central Japan

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Abstract

Euglena species were observed accompanying a filamentous green alga *Klebsormidium* community near a volcanic acidified spring in the southern part of Mt. Ontake, central Japan on 16 September 2016 (pH 3.6) and 25 November 2017 (pH 2.9). Rather than swimming, the *Euglena* actively expanded and contracted around the filaments of *Klebsormidium*. The *Euglena* cells were spindle-shaped with protruding tails. No flagella were observed. The inside of the cells contained numerous small, short rod-shaped paramylon grains surrounded by plate-like chloroplasts. Gaps were observed at the head, middle and tail of the cells. The head had a brilliant crimson red eyespot. In the elongated state, the cells were 40–90 µm long and 7–14 µm wide. Based on these cell morphological characteristics, the alga was identified as the acidophilic species *Euglena mutabilis*.

Key words: *Euglena mutabilis*, *Klebsormidium*, volcanic acidified spring, Mt. Ontake

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Introduction

An Euglenophyceae *Euglena mutabilis* Schmitz 1884 is a cosmopolitan species distributed in highly acidified freshwater environments (Wolowski, 2011). This species was commonly observed at artificially acidified mine drainage in the United Kingdom (Hargreaves *et al.*, 1975), the United States (Brake *et al.*, 2001), Spain (Sabater *et al.*, 2003), France (Casiot *et al.*, 2004), Portugal (Valente and Gomes, 2007) and Japan (Yanagawa *et al.*, 2021). For example, in acid mine drainage (<pH 3) in England, *E. mutabilis* was the most widespread species and often the most abundant, sometimes over 80 % of all species (Hargreaves *et al.*, 1975). *E. mutabilis* has also been observed at acidic volcanic streams and springs in the northern, north-eastern, eastern and south-western regions of Japan (Negoro, 1943; Negoro, 1944; Negoro, 1962; Yamagishi, 1977; Yanagawa *et al.*, 2021).

However, few algal flora surveys have been conducted in the volcanic areas of central Japan, representing an information gap. In addition, previous studies have not recorded *E. mutabilis* at volcanic hot springs or cold springs (Emoto and Yoneda, 1942; Yoneda, 1942a; Yoneda, 1942b), or at volcanic acidified streams (Nozaki, 2016; Nozaki *et al.*, 2020). Herein, we report on the appearance of *E. mutabilis* in a filamentous green alga *Klebsormidium* community near a volcanic acidified spring in the southern part of Mt. Ontake on 16 September 2016 and 25 November 2017. This study describes the cell morphology and habitat characteristics of *E. mutabilis*.

Methods

The location map and photos of the study site are shown in Figure 1. The sampling station was located in the southern part of Mt. Ontake, a volcano in central Japan (35°50′01″ N, 137°28′13″E, elevation 1270 meters). Algal and water samples were collected from a spring discharging into

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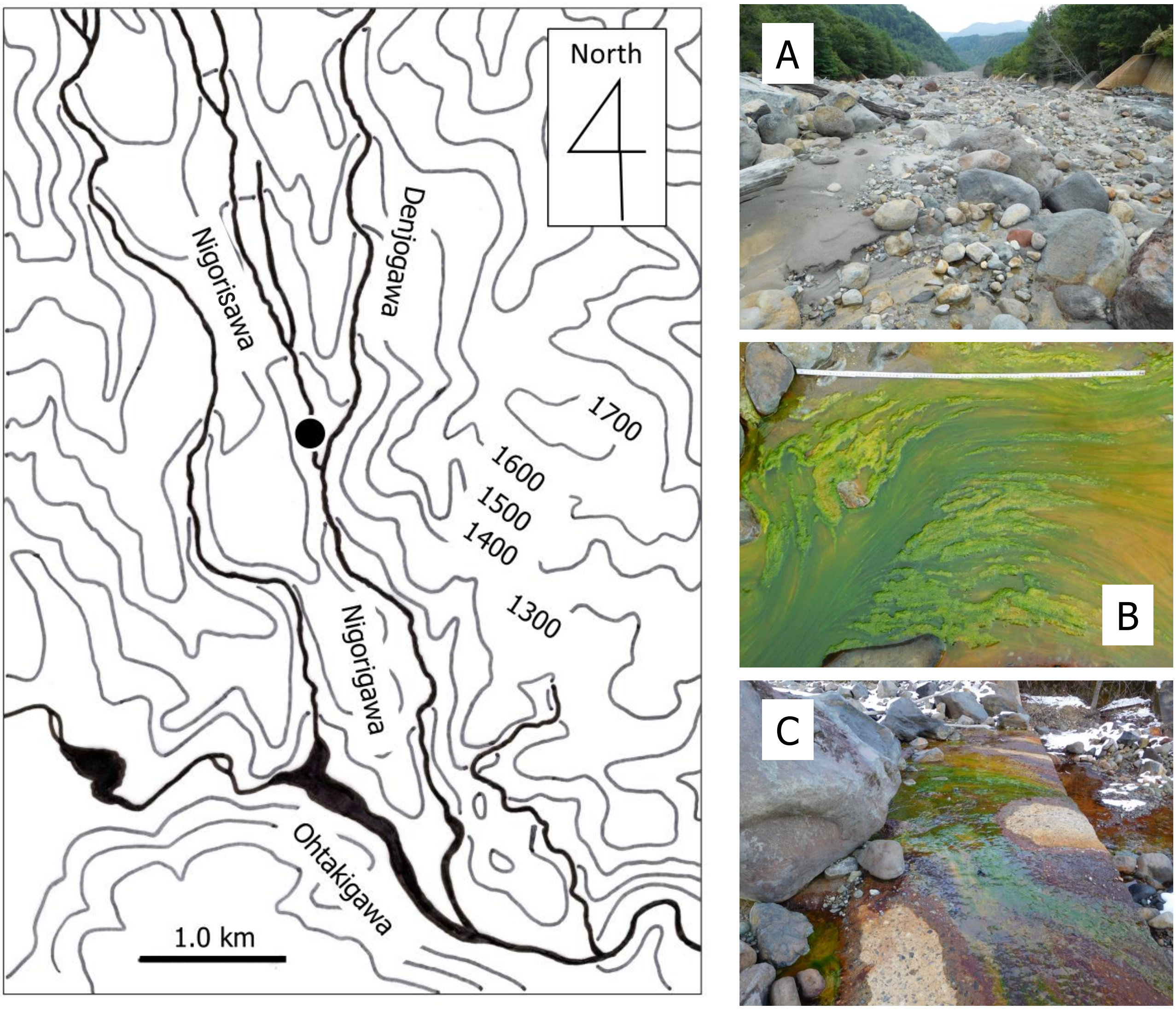

Figure 1. Location map and photographs of the study site. (A) Nigorisawa River on 16 September 2016. (B) *Klebsormidium* community seen propagating near the discharging volcanic acidified spring on 16 September 2016. (C) *Klebsormidium* community on 17 November 2017.

the channel of the Nigorisawa River, a volcanic sulfuric acidified stream flowing from a volcanic fumarole in the Jigokudani Valley, Mt. Ontake (Matsumoto *et al.*, 2020). The water temperature, pH (WAK-pH; KYORITASU-RIKA Co.) and electrical conductivity (CM21P; TOA-DKK) of the stream were measured at the sampling station. The algal and water samples were stored in a box with ice and sent to the laboratory within 24 hours of sampling.

The algal samples were observed under an optical microscope (BX51; OLYMPUS Co.), and photomicrographs were taken with a digital camera (DP27; OLYMPUS Co.). The width and length of the *Euglena* cells in the fresh samples were measured using software (cellSens Standard; OLYMPUS Co.). Specimens were donated to the Department of Botany, National Museum of Nature and Science (TNS-AL-63146 and TNS-AL-63148).

The turbidity of the raw water samples was measured using a water analyzer (WA1; NIPPON DENSHOKU Co.). The water samples were filtered through a glass fibre filter (GF-75; ADVANTEC Co.). The colour of the filtered water samples was also measured using a water analyzer (WA1; NIPPON DENSHOKU Co.). The nutrient concentration (NH_4^+-N, NO_2^--N, NO_3^--N and PO_4^{3-}-P) of the filtered water samples was analyzed using a spectrophotometer (UV-1280; SHIMADZU Co.). The nutrient analysis procedures followed those of Matsumoto and Nozaki (2014).

Results and Discussion

Figure 2 shows the photomicrographs of the *E. mutabilis*

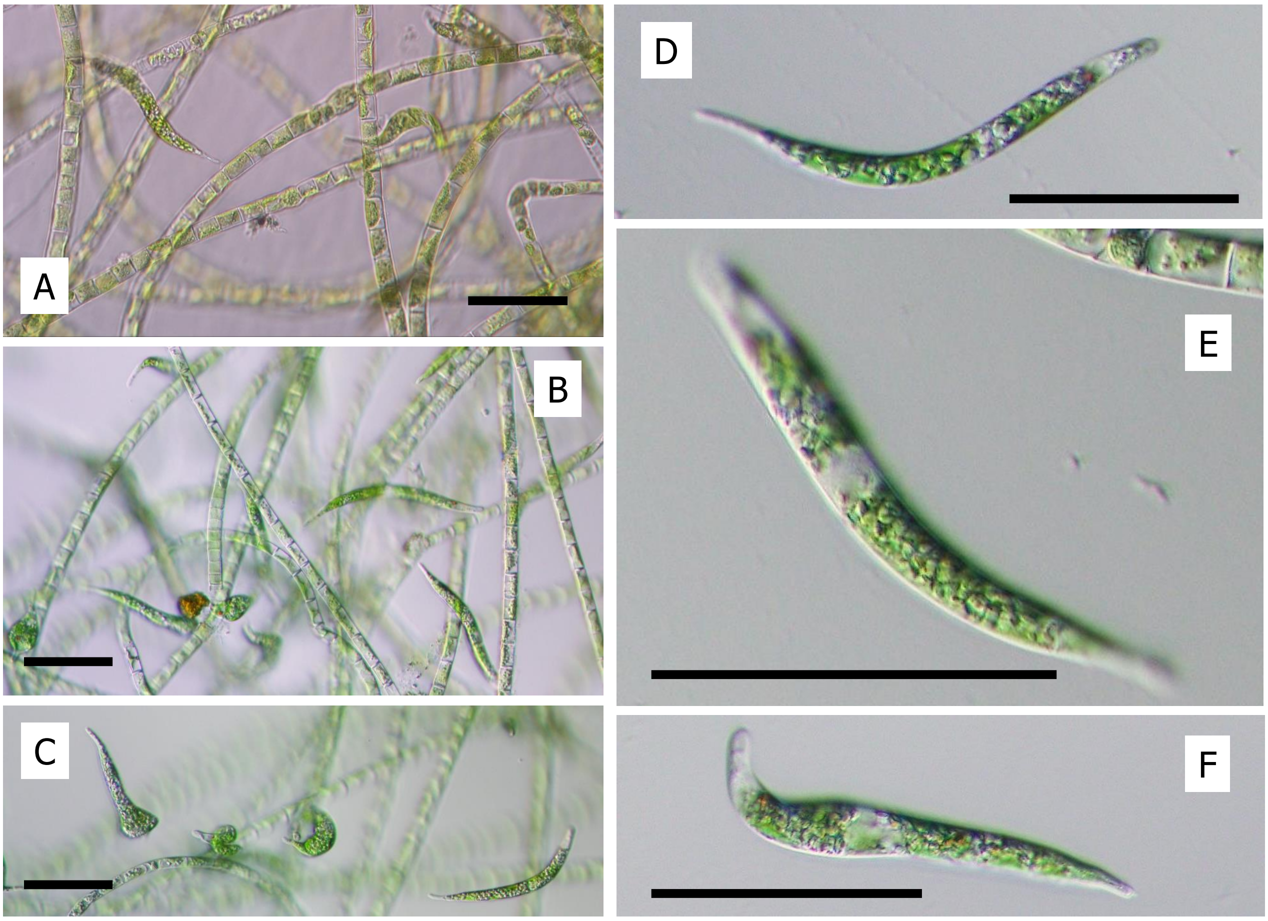

Figure 2. Photomicrographs of *Euglena mutabilis* collected from volcanic acidified spring water in the southern part of Mt. Ontake, central Japan on（A）16 September 2016 and（B-F）26 November 2017.（A-C）*E. mutabilis* in a filamentous green alga *Klebsormidium* community.（D-F）Elongated states of the *E. mutabilis* cells. Scale bars = 50 µm.

collected from the sampling station. This *Euglena* did not swim but instead actively expanded and contracted around the filaments of *Klebsormidium* (Figure 2A-2C). The cells were spindle-shaped with protruding tails. No flagella were observed. The inside of the cells contained numerous small, short rod-shaped paramylon grains surrounded by plate-like chloroplasts. Gaps were observed between the head, middle and tail of the cells. The head had a brilliant crimson red eyespot (Figure 2D-2F). In the elongated state, the cells were 40–90 µm long and 7–14 µm wide. These morphological characteristics are almost consistent with those described by Negoro (1943), Yamagishi (1977) and Wolowski (2011) for *E. mutabilis*. Thus, the alga was identified as the acidophilic species *E. mutabilis*.

The physical and chemical conditions of the volcanic acidified spring water at the sampling station are shown in Table 1. The temperature of spring water is generally influenced by air and soil temperatures (Arai, 2004). The air temperature measured on the Kaida-Kogen Highland (35°56′03″N, 137°36′01″E, elevation 1130 meters) near the sampling station was 19.9℃ at 12:50 on 16 September 2016 (daily average 16.3℃), and 1.2℃ at 16:00 on 25 November 2017 (daily average -1.6℃) (data retrieved from the Japan Meteorological Agency web site on 1 December 2024, https://www.jma.go.jp/jma/indexe.html). Although

Table 1. Physical and chemical conditions of the spring water at the sampling site.

	16 September 2016	25 November 2017
Sampling time	12:47	16:00
Water temperature（℃）	16.6	8.6
pH	3.6	2.9
Electrical conductivity（mS m^{-1}）	157.1	64.3
NH_4^+-N（µgN L^{-1}）	42	54
NO_2^--N（µgN L^{-1}）	25	no detect
NO_3^--N（µgN L^{-1}）	3	83
Dissolved inorganic nitrogen（µgN L^{-1}）	70	136
PO_4^{3-}-P（µgP L^{-1}）	63	97

Table 2. Records of *Euglena mutabilis* in Japan.

Location	Site characteristics	Date	Water temperature (℃)	pH	Accompanying alage	Reference
Kawayu-onsen, Hokkaido	Hot spring	21 July 1941	42.0	1.0	no description	Negoro (1943); Negoro (1944)
Mt. Osorezan, Aomori	Hot spring drainage	18 July 1941	21.0	2.9	*Cyanidium caldarium, Pinnularia braunii*, *P. acoricola, Eunotia septentrionalis*, *E. osoresanensis*, *Hormidium vulcanum, Zygnema* sp.	Negoro (1943); Negoro (1944)
Sukayu-onsen, Mt. Hakkouda, Aomori	Acidified stream	26 July 1940	19.5 - 20.8	2.9	*Pinnularia braunii* var. *amphicephala*	Negoro (1943); Negoro (1944)
Katanuma, Naruko, Miyagi	Acidified lake	21 June 1941	23.0	1.7	*Cyanidium caldarium, Pinnularia braunii* var. *amphicephala, Hormidium vulcanum*	Negoro (1943); Negoro (1944)
Numajiri sulfur mine, Fukushima	Mine drainage	29 August 1940	18.1	2.9	*Pinnularia braunii* var. *amphicephala*	Negoro (1943); Negoro (1944)
Yumoto-onsen, Nikkou, Tochigi	Acidified stream	30 November 1941	21.3	3.0	no description	Negoro (1943); Negoro (1944)
Ohwakudani, Mt. Hakone, Kanagawa	Head stream near volcanic fumarole	28 May 1939	22.2	2.9	*Pinnularia braunii* var. *amphicephala*	Negoro (1943); Negoro (1944)
		16 August 1940	26.0 - 31.8	2.9 - 3.0		
		16 September 1941	16.5	2.7		
		4 October 1942		2.8		
Jyuman jigoku-onsen, Beppu, Ohita	Hot spring	22 August 1941	30.9 - 35.0	3.0	no description	Negoro (1943); Negoro (1944)
Sensui coal mine, Kurate, Fukuoka	Mine drainage	26 April 2019	22.5	4.1	no accompayning	Yanagawa *et al.* (2021)
Bougatsuru mire, Takeda, Ohita	Cold spring	28 November 2019	10.2	4.8	fibrous cyanobacteria	Yanagawa *et al.* (2021)
Ebino-kogen, Mt. Kirishima, Miyazaki	Temporal pond	17 May 2019	16.2	3.4	fibrous cyanobacteria	Yanagawa *et al.* (2021)

the spring water temperature (16.6 ℃) at the sampling station in September 2016 was somewhat lower than the corresponding air temperature, it was close to the September daily average air temperature. However, the spring water temperature in November (8.6℃) was higher than the air temperature. The water temperature during September was likely increased by direct exposure to solar radiation (Arai, 2004) due to the sampling station not being covered by vegetation (Figure 1A).

The 50 cm deep soil temperature at the sampling station was estimated using the following regression coefficients obtained from investigations in the southeast part of Mt. Ontake by Sekine *et al.* (1984) as follows:

September $T = - 0.0038H + 19.8$
November $T = - 0.0076H + 25.3$
where T is the soil temperature at 50 cm deep (℃), and H is the elevation (1270 meters).

The 50 cm deep estimated soil temperatures are 15.0℃ for September 2016 and 15.7℃ for November 2017. The water temperature in November 2017 was lower than the corresponding estimated soil temperature. Therefore, the spring water flows underground at a layer of shallower than 50 cm deep, it is thought to be largely influenced by seasonal changes in air temperature.

The water chemistry was characterized as acidic with a high inorganic matter concentration based on the pH and electrical conductivity. Matsumoto *et al.* (2020) reported a pH of 3.7 and an electrical conductivity of 36.7 mS m^{-1} on 25 and 26 November 2017 in the main channel of the Nigorisawa River located near the sampling station. Because the spring water flows through the riverbed sediments of the Nigorisawa River, the electrical conductivity of the spring water may be higher than the stream water because of the addition of the inorganic matter. The concentrations of dissolved inorganic nitrogen in the spring water are about the same as those recorded in the downstream of the Nigorisawa River, however, the concentrations of PO_4^{3-}-P were markedly high (Nozaki, 2016). Since the concentration of NH_4^+-N was also higher than that of the stream water, the spring water chemistry appears to form in an underground low-oxygen environment.

Records of occurrences of *E. mutabilis* in Japan are shown in Table 2. The following algae have been recorded accompanying *E. mutabilis*: a red algae, *Cyanidium caldarium*; pennate diatoms, *Eunotia* and *Pinnularia*; filamentous green algae, *Hormidium vulcanum* and *Zygnema*; and fibrous cyanobacteria (Negoro, 1943; Negoro, 1944; Yanagawa *et al.*, 2021). *E. mutabilis* is recorded as often been appearing with *Pinnularia braunii* var. *amphicephala* (Negoro, 1943; Negoro, 1944). *P. braunii* var. *amphicephala* is currently identified as *P. acidojapnica* (Idei and Mayama, 2001). *Pinnularia* and a filamentous green alga *Klebsormidium* also appear with *E. mutabilis* in acid mine drainage in Spain and Portugal (Sabater *et al.*, 2003; Valente and Gomes, 2007). The current study reports the first observation of *E. mutabilis* accompanying *Klebsormidium* in Japan.

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摘　要

御嶽山南麓の無機酸性湧水における糸状緑藻 *Klebsormidium* 群落内でのミドリムシ *Euglena mutabilis* の出現

野崎健太郎

御嶽山南麓の火山性の酸性湧水において，2016年9月16日（pH 3.6），2017年11月25日（pH 2.9）に糸状緑藻 *Klebsormidium* 属の群落内でミドリムシが出現した。このミドリムシは，遊泳せず *Klebsormidium* の糸状体の周囲で盛んに伸縮運動を行っていた。細胞の形状は紡錘形で尾部は突起状を示した。鞭毛は観察できなかった。細胞内部には，小さな短棹状のパラミロン粒が多数含まれ，それらを板状の葉緑体が包み，細胞の中間部および頭部と尾部には空隙が見られた。頭部には暗赤色の明瞭な眼点が存在する。伸びた状態での細胞の長さは40〜90 µm，幅は7〜14 µm であった。これらの特徴から，このミドリムシは好酸性種の *Euglena mutabilis* と同定された。

Appearance of *Euglena mutabilis* with a filamentous green alga *Klebsormidium* community

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