

Phenology and Morphology of the Two Freshwater Red Algae (Rhodophyta) in the Imperial Palace, Tokyo

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Abstract. Phenological and morphological observations of the two freshwater red algae (Florideophyceae, Rhodophyta), *Batrachospermum atrum* (Hudson) Harvey (Batrachospermaceae, Batrachospermales) and *Hildenbrandia rivularis* (Liebmann) J. Agardh (Hildenbrandiaceae, Hildenbrandiales) were carried out in the Imperial Palace, Tokyo from May 2011 to August 2012. The existence of these two species indicates that water environment inside the Imperial Palace is protected against the polluted fluid from the urban area of Tokyo. *Batrachospermum atrum* developed gametophytes (*Batrachospermum*-stage) only from April to June, and then produced *Audouinella*-like monosporophytes (*Audouinella*-stage) in November and March in the water of the Dokan Moats. *Hildenbrandia rivularis* continued living as red crustous thalli (putative sporophytes) without sexual reproduction on the surface of stones in the little stream inside of the Fukiage Garden throughout the year.

Key words: *Audouinella*-stage, *Batrachospermum atrum*, *Hildenbrandia rivularis*, Imperial Palace, Rhodophyta, Tokyo.

Introduction

Most of the freshwater macroalgal red algae (Florideophyceae, Rhodophyta) inhabit unpolluted clean inland waters throughout the world, therefore they can be used as indicators for quality of waters. In other words, the extinction of the red algae in the inland waters means loss of clean waters caused by pollution mainly by human activities (Kumano, 2002). The Imperial Palace, which is located in the heart of urban area in Tokyo, is famous for its enriched biota consisted of fauna and flora that are distinct from the outside area. In the previous investigations carried out during 1995–1999, three freshwater red algae were collected from the inside of the Imperial Palace (Watanabe, 2000): *Audouinella* sp. (Acrochaetia-ceae, Acrochaetiales), *Batrachospermum atrum* (Hudson) Harvey (Batrachospermaceae, Batrachospermales), *Hildenbrandia rivularis* (Liebmann) J. Agardh (Hildenbrandiaceae, Hildenbrandiales).

However, in the preliminary investigations made by the author in 2010, only *H. rivularis* was observed, while other species were not found in any places of collecting sites recorded in the previous report by Watanabe (2000).

In general, it is necessary to investigate the field at all seasons for making its macroalgal flora clear, because most macroalgae repeat an alternation of generations between macroscopic stage and microscopic stage every year, which means that some algae are invisible to the naked eye during several months in a year. Thus, in this study, to confirm their presence and seasonal changes in the Imperial Palace, the two freshwater red algae, *Batrachospermum atrum* and *Hildenbrandia rivularis*, were observed and collected every month for a year from May 2011 to August 2012.

Materials and Methods

The freshwater red algal materials referable to

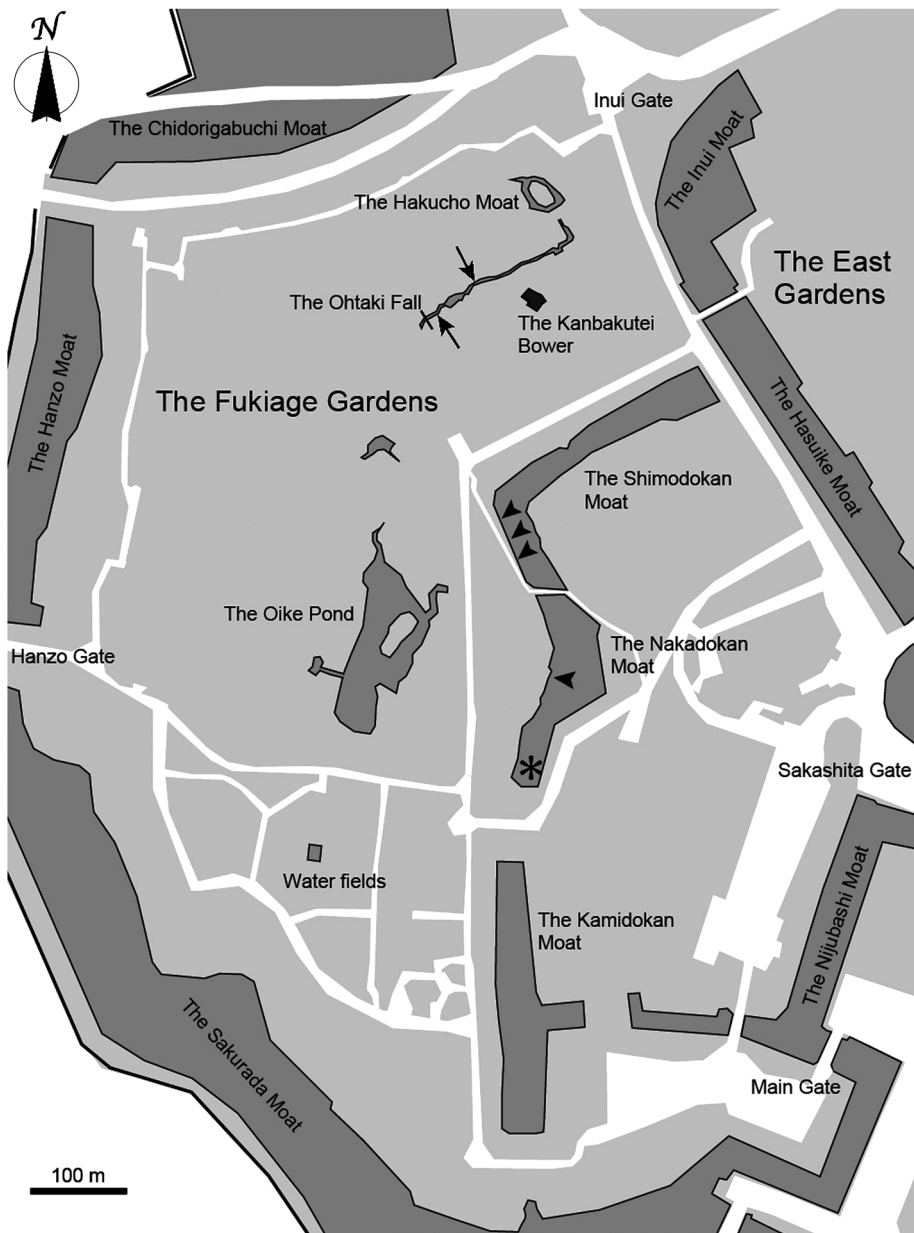


Fig. 1. Map of investigation sites in the Imperial Palace, Tokyo, showing the habitats of *Batrachospermum atrum* (arrowheads) and *Hildenbrandia rivularis* (arrows). The asterisk indicates the locality where *B. atrum* was collected in 2000.

Batrachospermum atrum and *Hildenbrandia rivularis* were observed and collected once a month in the Imperial Palace, Tokyo from May 2011 to August 2012: 30 May 2011, 27 June 2011, 25 July 2011, 22 August 2011, 26 September 2011, 31 October 2011, 28 November 2011, 26 December

2011, 30 January 2012, 27 February 2012, 26 March 2012, 16 April 2012, 28 May, 2012, 25 June 2012, 30 July 2012, 27 August 2012. In each investigation water temperature was measured directly at the stream near the Kanbakutei Bower in the Fukiage Gardens and at the west coast of

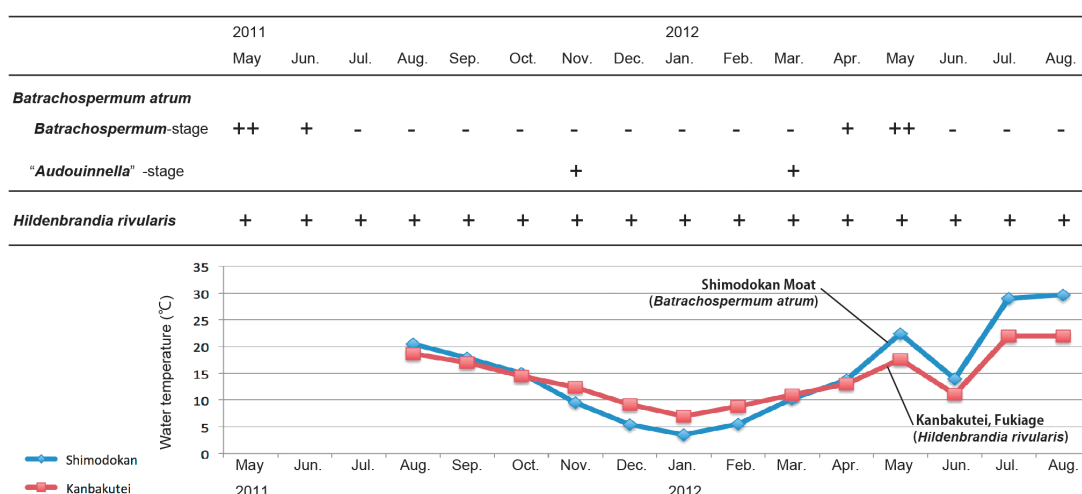


Fig. 2. Phenology of *Batrachospermum atrum* and *Hildenbrandia rivularis* with monthly changes of water temperatures in the Shimodokan Moat and the stream near the Kanbakutei in the Fukiage Gardens, the Imperial Palace, Tokyo.

Shimodokan Moat (Fig. 1). The living materials were carried to the laboratory and observed anatomically using a microscope. For preservation, the material was dried on sheets of paper or fixed in 10% Formalin-seawater. Voucher specimens were deposited in the algal herbarium of the National Museum of Nature and Science, Tsukuba, Japan (TNS).

Results

Phenology of the two freshwater red algae and seasonal changes of the waters in their habitats are shown in Fig. 2. *Batrachospermum atrum* was found several times after careful searching in the Dokanbori Moats (Fig. 3), while *Hildenbrandia rivularis* was easily collected through the year from the little stream inside the Fukiage Imperial Gardens. *Batrachospermum atrum* appeared as two kinds of thalli in the same place (Fig. 1, arrowheads) in different seasons: from April to June gametophytic thalli (*Batrachospermum*-stage), and then in November and March sporophytic thalli ("*Audouinella*"-stage) grew. In half of the year, however, the author could not find any thalli of this species. On the other hand, *Hildenbrandia rivularis* was so conspicuous that

the author could collect it at the same places every time (Fig. 1 arrows) throughout the year (Fig. 2), because the red or crimson thalli painted the bottom of the little stream dark red. Morphology and taxonomy of the two algae are as follows:

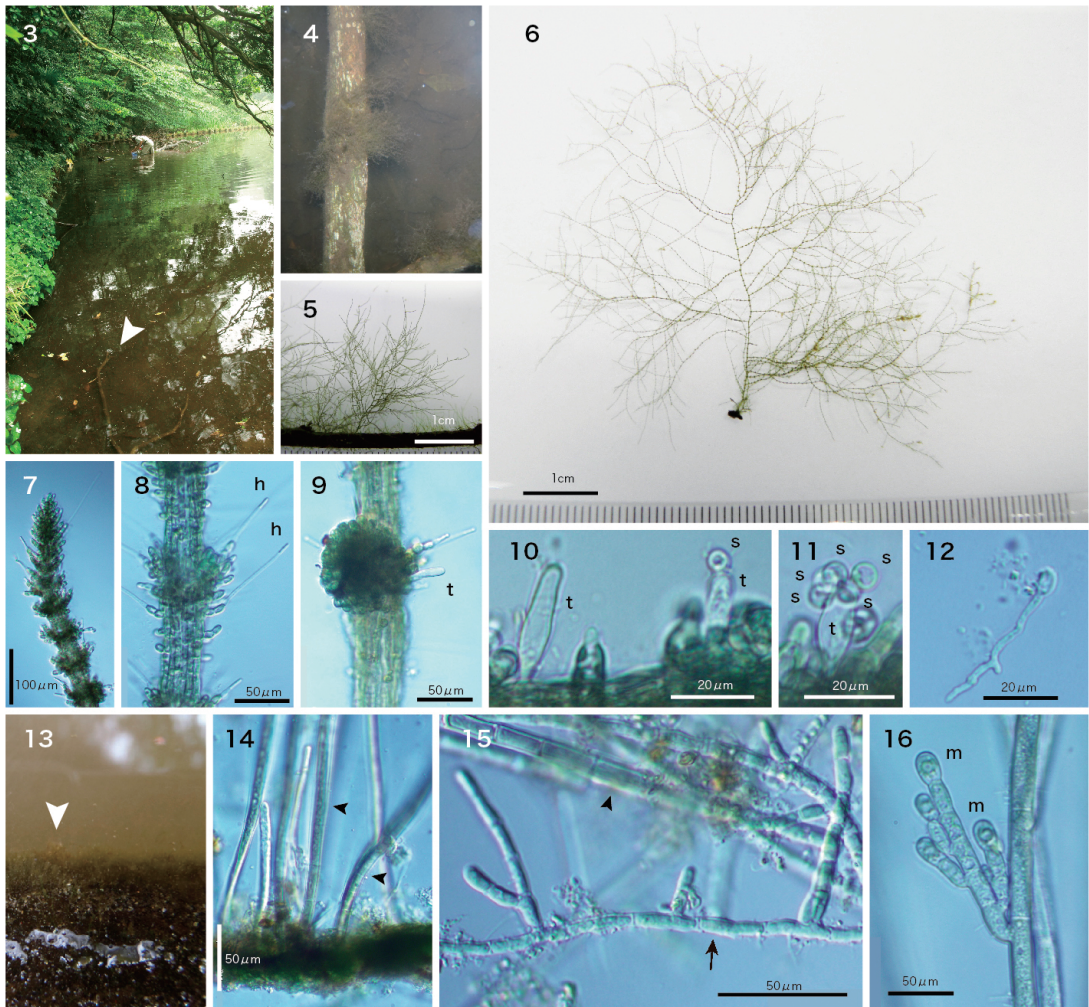
Order Batrachospermales Pueschel & Cole, 1982
Family Batrachospermaceae C. Agardh, 1824

1. ***Batrachospermum atrum*** (Hudson) Harvey in *A Manual of the British Algae...*: 120 (1841).
Basionym: *Conferva atra* Hudson, *Flora Angelica...*: 597 (1778).

Synonyms: *Batrachospermum tenuissimum* (Bory de Saint-Vincent) Duby, *B. dillenii* Sirodot, *B. gallae* Sirodot, *Sirodotia angolensis* (W. West & W. G. West) Skuja (Kumano, 2002).

[Figs. 4–16]

Batrachospermum-stage: Thalli are erect, up to 7 cm in height, densely tufted, composed of numerous filaments, epilithic or attached to dead or fallen tree branches (Fig. 4), green to blue-green in colour (Fig. 5). The thalli are ramified irregularly (Fig. 6). The filaments are cylindrical, 40–145 μm in diameter, composed of a uniseriate central axis and many cortical filaments covering the axis



Figs. 3–16. *Batrachospermum atrum* from the Imperial Palace, Tokyo. 3. Habitat in the Shimodokan Moat, showing a fallen tree branch, which is a substratum for the alga (arrowhead). This habitat is protected from the direct rays of the sun by trees. 4–11. *Batrachospermum*-stage. 4–5. Habit. Gametophytic thalli on the tree branches. 6. Gametophytic thallus before making the dried specimen (TNS-AL 183413). 7. Apex of a thallus with young whorls. 8. Axis with young whorls and many terminal hairs (h) protruding from young fascicles. 9. Carposporophyte on an axis, showing a trichogyne (t). 10. Unfertilized trichogyne and fertilized trichogyne with a spermatium (s). 11. Fertilized trichogyne with five spermatia. 12–16. “*Audouinella*”-stage. 12. Germination of a carpospore. 13. *Audouinella*-like thalli (arrowhead) on the surface of tree branch. 14. *Audouinella*-like erect filaments (arrowhead) on the substratum. 15. *Audouinella*-like erect filaments (arrowhead) and prostrate filaments (arrow). 16. Monosporangia (m) on the terminal of the laterals.

(Figs. 7–9). Whorls are compact and small, conical to ovoid, composed of short fascicles with several cells (Fig. 8). Terminal hairs are abundant (Fig. 8, h). Plants are monoecious. Spermatangia are spherical and terminal on fascicles. Trichogynes are ellipsoidal to inflated-ovoid (Figs. 9–10, t).

Fertilizations are observed frequently (Figs. 10, 11). Carposporophytes are hemispherical, composed of short carpogonium-bearing branches (Fig. 9). Carpospores are spherical and protruded rhizoidal filaments after germination (Fig. 12).

“*Audouinella*”-stage: Thalli are tufted, up to

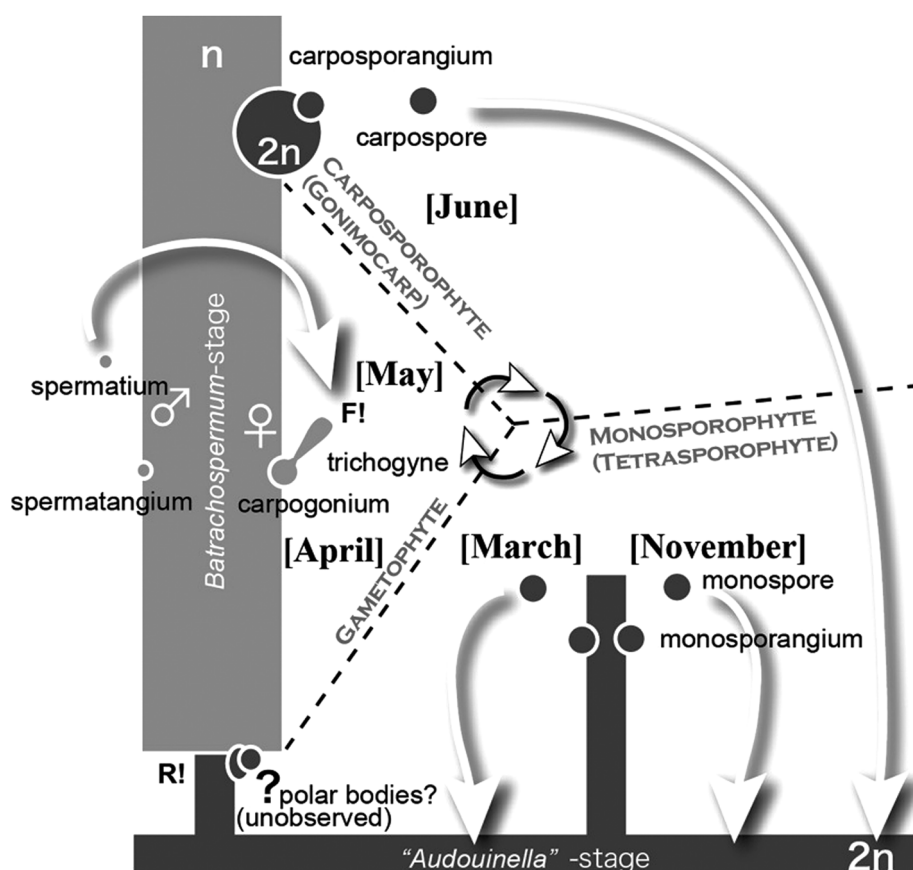


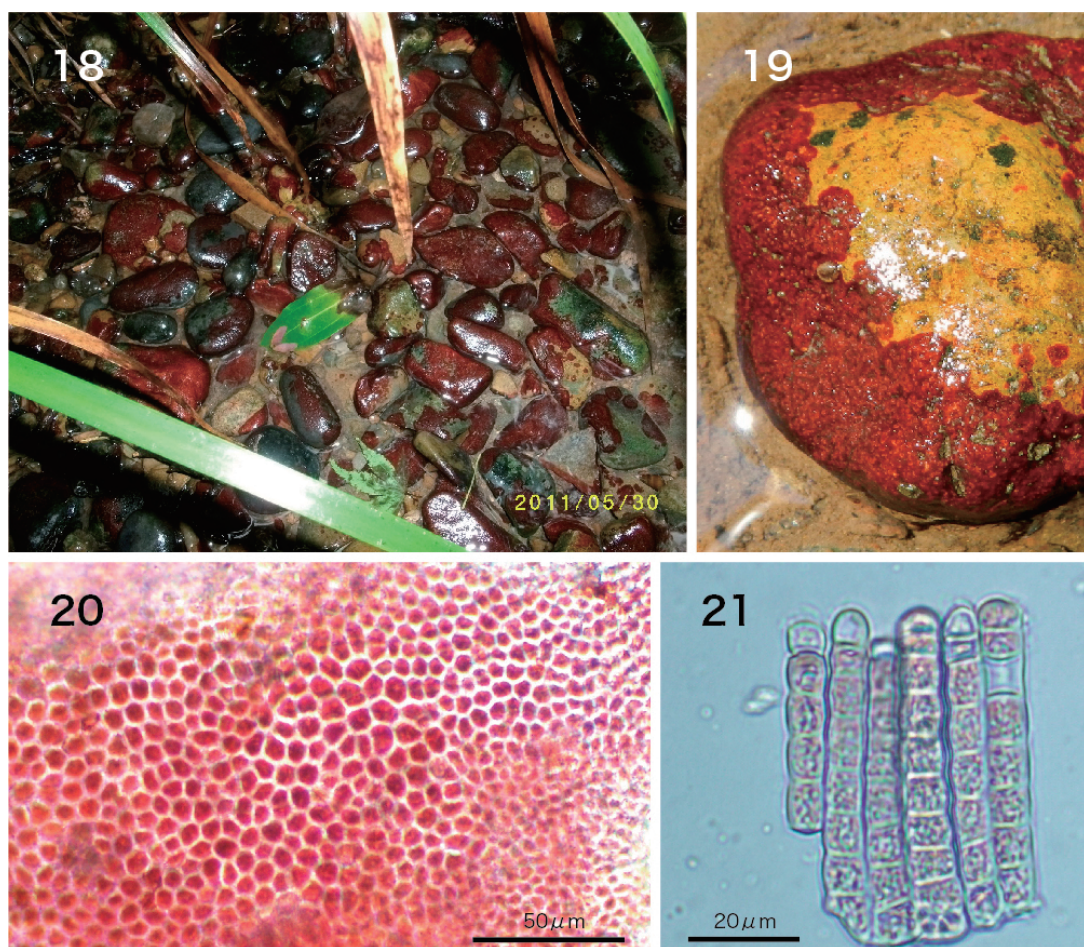
Fig. 17. An estimated life cycle of *Batrachospermum atrum* in the Imperial Palace, Tokyo. F!: fertilization, R!: meiosis, n: haploid, 2n: diploid, ?: polar bodies were not observed in this study.

6 mm in height, attached to dead or fallen tree branches (Fig. 13, arrowhead). The thalli are blue-green in color, composed of erect, straight filaments (Fig. 14, arrowheads, Fig. 15, arrowhead) and prostrate, rhizoidal filaments (Fig. 15, arrow). Monosporangia are ellipsoidal, on the terminal of the laterals of the erect filaments (Fig. 16, m).

Specimens examined: The Dokan Moats, the Imperial Palace, Tokyo, leg. T. Kitayama: the southwest coast of the Shimodokan Moat (35°41'05"N, 139°45'04"E), 30 May 2011 (TNS-AL 183411, 183412), 16 Apr. 2012 (TNS-AL 183413, Fig. 6); the west coast of the Nakadokan Moat (35°41'01"N, 139°45'05"E), 28 May 2012 (TNS-AL 183414).

Distribution: Cosmopolitan (Kumano, 2002; Guiry and Guiry, 2013).

Remarks: It is not easy to find the existence of this species in any localities by a short limited period of investigation because the alga has a unique heteromorphic life cycle with alternation of three generations: a macroscopic gametophyte, a microscopic carposporophyte (or gonimocarp) which is parasitic on the gametophyte, and microscopic monosporophyte (or tetrasporophyte) (Fig. 17). In place of 'gonimocarp' the author uses temporarily the term 'carposporophyte', which means an independent phase of the life cycle. However, this diploid stage is not considered recently as an independent phase, because there is no report about a red alga with an autonomous 'carposporophyte' and no reason to be regarded as equivalent to the gametophyte or the tetrasporophyte (van den Hoek 1995). The author also uses the



Figs. 18–21. *Hildenbrandia rivularis* (Liebmann) J. Agardh from the Imperial Palace, Tokyo. 18. Habitat in the little stream near the Kanbakutei Bower, the Fukiage Gardens, showing many dark-red pebbles covered with the crustose thalli on the shallow bottom. 19. Crimson thalli on the stone. 20. Surface view of the thallus. 21. Transverse view of the cross section of the thallus showing the erect filaments.

term ‘monosporophyte’ for “*Audouinella*”-stage instead of ‘tetrasporophyte’, which is used for this stage in the most of red algae, because this alga has no tetrasporangia.

In the Imperial Palace, it is estimated that an annual life cycle by three generations is performed as follows. The haploid gametophyte (*Batrachospermum*-stage) appears in April and carries out fertilization by a spermatium (male gamete lacking flagella) and carposonium with a trichogyne until June. Carposonium after fertilization produces diploid filaments (gonimoblasts), which form a carposporophyte on the

gametophyte and release carpospores from its carposporangium. The carpospore develops into a diploid monosporophyte (“*Audouinella*”-stage), which produces monosporangia in November and March and releases monospores for asexual propagation. In April the monosporophyte produces new gametophytes directly on the erect filaments after meiosis (the author could not confirm it). As a result, all of the three generations are unified as an individual on the life cycle (Fig. 17).

This is the first report on “*Audouinella*”-stage (or “*Chantransia*”-stage) of this species.

Order Hildenbrandiales
Family Hildenbrandiaceae

2. **Hildenbrandia rivularis** (Liebmann) J. Agardh

in Species Genera et Ordines Algarum...: 379 (1851).

Basionym: *Erythroclathrus rivularis* Liebmann, Naturhistorisk Tidsskrift **2**(1): 174 (1893).

[Figs. 18–21]

Thalli are prostrate, circular discoid to crustose, covering the surface of pebbles or stones, red to crimson in colour (Figs. 18, 19). The thalli are consisting of thin basal layers and short erect filaments. Erect filaments are uniseriate, aggregated densely (Figs. 20, 21). Reproductive organs were not observed.

Specimens examined: The little stream near the Kanbakutei Bower, the Fukiage Gardens, Imperial Palace, Tokyo (35°41'14"N, 139°45'02"E), leg. T. Kitayama: 30 May 2011 (TNS-AL 183408), 22 August 2011 (TNS-AL 183409), 28 November 2011 (TNS-AL 183410).

Distribution: Cosmopolitan (Kumano, 2002; Guiry and Guiry, 2013).

Remarks: This species is considered to have an ability of asexual propagation by fragmentation of erect filaments, which are the putative sporophytes (Seto, 1993).

Discussion

The existence of the two freshwater red algae indicates that the water environment is maintained in the Imperial Palace, Tokyo. It is prevented from contamination of waste water from outside the circular moats during the last decade. *Batrachospermum atrum* and *Hildenbrandia rivularis* are known to require not only unpolluted water but also low water temperature and movement of water (Kumano, 2002). The water in their habitats is kept at relatively low temperature (Fig. 2) because the water of the stream, where *H. rivularis* grows, is supplied from the groundwater under the Fukiage Gardens, and then flows into the Dokan Moats, where *B. atrum*

appears occasionally.

In the previous investigation during 1995–1999, *Batrachospermum atrum* was collected from the southernmost area for once (Watanabe, 2000). However, the author could not find any thalli of *Batrachospermum* in the locality (Fig. 1, asterisk) because the water area was covered with thick mud recently. It is uncertain whether the habitat in the area had been lost or not.

B. atrum has a unique life cycle by heteromorphic alternation of generations, which macroscopic gametophyte (*Batrachospermum*-stage) appears only from April to June in the Imperial Place. Possibly day length also has an influence on development of gametophytes of *B. atrum*, whose habitats are protected against the direct sun rays by trees (Fig. 3). Thus there is also a possibility that the localities of appearance of gametophytes change depending on the season. It is important to investigate more frequently in various seasons in order to grasp the actual situation of the freshwater red algal flora in the Imperial Palace.

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皇居の淡水産紅藻2種の季節的消長と形態について

北山太樹

2011年5月から2012年8月にかけて、皇居において淡水産紅藻2種、イシカワモズク *Batrachospermum atrum* (Hudson) Harvey (カワモズク目カワモズク科) とタンスイベニマダラ *Hildenbrandia rivularis* (Liebmann) J. Agardh (ベニマダラ目ベニマダラ科) の季節的消長と形態を観察した。なお、これら2種は人為的な汚染に弱いため都市環境には出現せず、環境省によって絶滅危惧種に指定されている藻種でもあるが、皇居では前回の調査で採集されたのに引き続き、10年以上を経た今回の調査でも生育していることが確認できた。このことは皇居の水環境が都市部からの排水の影響を受けていないことを示唆する。2011年5月から2012年8月にかけての周年観察の結果、イシカワモズクは4～6月に配偶体、3月と11月に孢子体が発見し、それぞれ旺盛な生殖活動が行なわれていることが明らかになった。一方、タンスイベニマダラは一年を通して生育が観察されたが、生殖器官を確認することはできなかった。