Takashi SUGAWARA*: **Further investigations on karyotype and C-banding pattern in seven species of *Heterotropa***

菅原 敬*: カンアオイ属数種の核型および C-分染型について

(Pl. I)

The genus *Heterotropa* occurs in north subtropical and temperate regions of Eastern Asia, especially of China and Japan. About 30 species have been known in Japan (Maekawa 1933, 1968), and they have more or less narrow distribution. Concerning the Japanese species of *Heterotropa*, karyological investigations have been carried out by several workers (Tanaka 1935, Ono 1960, Yuasa & Maekawa 1976, Sugawara 1981), and it has been reported that all the species examined have the same chromosome number 2n=24 except in a few cases of polyploid (Tanaka 1935, Ono 1960) and resemble one another also in karytoype. Furthermore, it was reported by the author (Sugawara 1981) that in their karyotypes one pair of small subtelocentric SAT-chromosomes was found commonly as a distinct chromosome, which exhibited a peculiar C-banding style. Based on these facts, he suggested that the Japanese species of *Heterotropa* may be a distinct group cytotaxonomically and may be characterized by the presence of one pair of the small subtelocentric SAT-chromosomes and by its peculiar banding style. However, further investigations on karyotypes and C-banding patterns in several species of the genus occurring in Shikoku and Kyushu districts revealed new karyological features, deviating from the above-mentioned characteristics. In this paper, therefore, I report the results of examination of karyotype and C-banding pattern in seven species of *Heterotropa* and discuss the relationships among these species from karyological ponints of view.

Materials and Methods All the materials examined in this study were collected from native habitats and they were transplanted into the garden of Makino Herbarium (MAK), Tokyo. The localities and the number of individuals examined are listed in Tab. 1. Meristem of root tips was used for analysis of karyotype and C-banding pattern. The cytological techniques applied here were

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Tab. 1. Chromosome numbers, localities and number of individuals examined.

Species	Chromosome number (2n)	Locality[1)]
Heterotropa		
H. aspera (F. Maekawa) F. Maekawa	24	Kodeishi (Kyoto Pref.)-10, Kannonji (Shiga Pref.)-2, Mt. Koya (Wakayama Pref.)-2
H. hexaloba var. *perfecta* F. Maekawa	24	Mt. Kurao (Kagoshima Pref.)-7, Mt. Odake (Kagoshima Pref.)-7
H. subglobosa F. Maekawa	24	Mt. Ichifusa (Kumamoto Pref.)-8
H. okinawensis (Hatusima) F. Maekawa	24	Mt. Katsuu-dake (Okinawa Pref.)-7
H. sakawana (Makino) F. Maekawa	24	Namekawa (Kochi Pref.)-2, Sakawa (Kochi Pref.)-6
H. costata F. Maekawa	24	Murotsu (Kochi Pref.)-9, Tsuro (Kochi Pref.)-5, Nahari (Kochi Pref.)-3
H. minamitania (Hatusima) F. Maekawa	24	Hyuga-shi (Miyazaki Pref.)-1

[1)] The number of individuals examined is inserted after a hyphen.

the same as those used in my previous report (Sugawara 1981). The materials are now under cultivation and will be preserved in MAK as dried voucher specimens.

Results

H. aspera and *H. hexaloba* var. *perfecta*. These two closely related species have the same chromosome number 2n=24 (Fig. 1A, B), congruent with previous reports (Tanaka 1935, Ono 1960), and their karyotypes and C-banding patterns were also similar each other. In each chromosomal complement (Fig. 2A, B), one pair of small subtelocentric SAT-chromosomes was found in common as a distinct chromosome. The subtelocentric SAT-chromosome of this type showed a peculiar C-banding style: the C-bands appeared both at the centromeric and at the distal regions of the short arm, but a satellite region lacks a heterochromatin (Pl. IA, B; Fig. 3A). Therefore, it was easily recognizable in a chromosomal complement. The other chromosomes were metacentric, of which four chromosomes were somewhat long in length. All the metacentric chromosomes

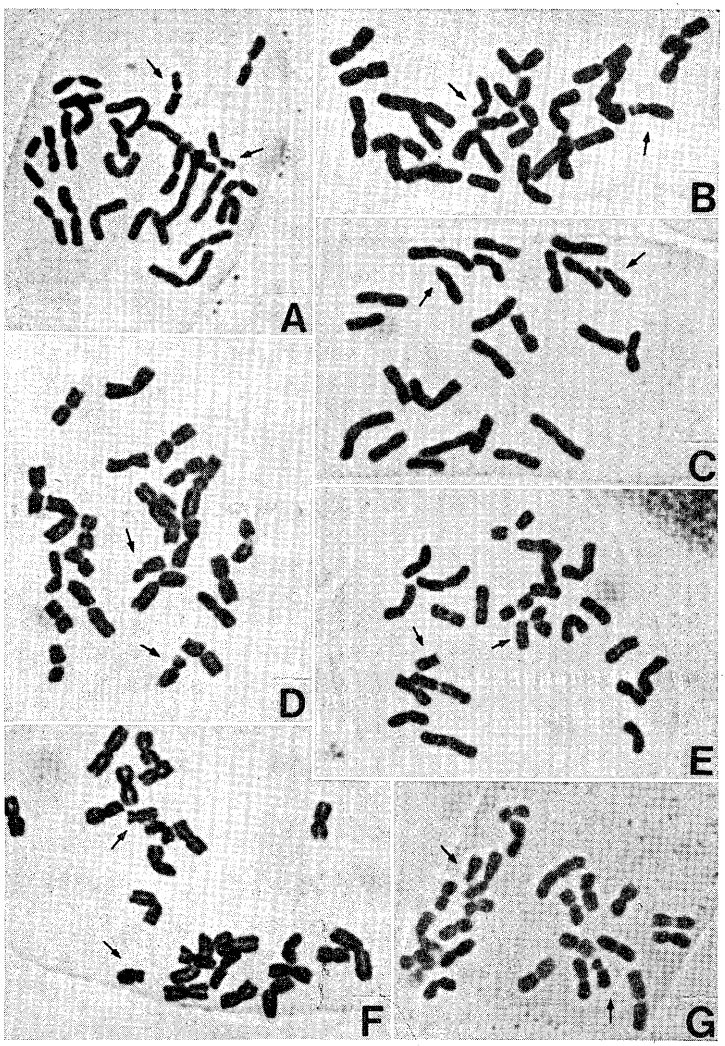

Fig. 1. Somatic metaphase chromosomes in seven species of *Heterotropa* (2n=24). A: *H. aspera*. B: *H. hexaloba* var. *perfecta*. C: *H. subglobosa*. D: *H. okinawensis*. E: *H. costata*. F: *H. minamitania*. G: *H. sakawana*. In A-D, arrows indicate the small subtelocentric SAT-chromosomes, while in E-G the small subtelocentric chromosomes. All: ×1500.

possessed centromeric C-bands which appeared either as a single band or as two dots (Pl. IA). In some cases, however, the centromeric C-bands were obscure or pale. In two metacentric chromosomes, a large C-band was observed at the centromeric region. This is a feature observed constantly in all preparations examined. Giemsa C-stained interphase nucleus showed numerous chromocenters which corresponded with the centromeric C-bands in metaphase chromosomes.

H. subglobosa. The somatic chromosome number was counted as 2n=24 for the first count to the species (Fig. 1C). The karyotype and C-banding pattern of this species are fairly similar to those of *H. aspera* and *H. hexaloba* var. *perfecta* stated above. Namely, the karyotype was represented by 11 pairs of metacentric chromosomes and one pair of small subtelocentric SAT-chromosomes (Fig. 2C). The subtelocentric SAT-chromosome was easily recognizable in a

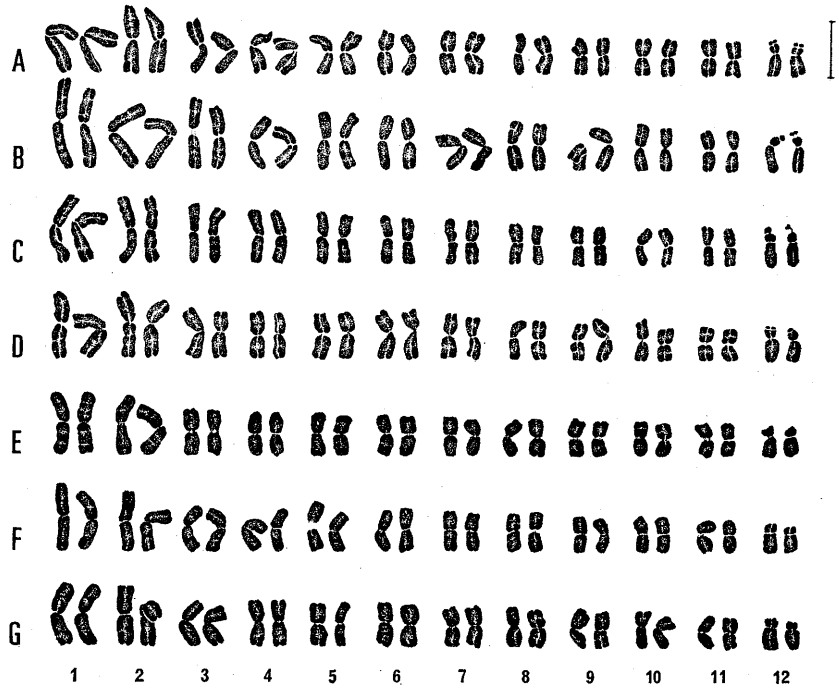

Fig. 2. Serial arrangement of the somatic metaphase chromosomes in seven species of *Heterotropa* (2n=24). A: *H. aspera.* B: *H. hexaloba* var. *perfecta.* C: *H. subglobosa.* D: *H. okinawensis.* E: *H. sakawana.* F: *H. costata.* G: *H. minamitania.* Bar: 5 μm.

chromosomal complement, since it showed a peculiar C-banding style as shown in Pl. IC. All the metacentric chromosomes possessed a intensive centromeric C-band. Additional intercalary C-bands were found in two metacentric chromosomes (Pl. IC).

H. okinawensis. The somatic chromosome number 2n=24 was counted in this species (Fig. 1D), congruent with a previous report (Yuasa & Maekawa 1976). The karyotype of this species was represented by 11 pairs of metacentric chromosomes and one pair of small subtelocentric SAT-chromosomes (Fig. 2D). In this karyogram, two metacentric pairs no. 1 and no. 2 were somewhat longer than the rest, and occasionally showed secondary constrictions on both arms. All the chromosomes showed C-bands (Pl. ID). In two metacentric chromosomes, the broad C-band was observed at the centromeric region. Intercalary C-bands were found in four metacentric chromosomes; viz, in two large ones, the C-band appeared as two dots near the distal region of the arm, and in the other two middle-sized ones appeared near the proximal region. In this species was also found one pair of the small subtelocentric SAT-chromosomes which exhibited C-bands at the centromeric and at the distal regions of the short arm, but a satellite region was negative to Giemsa staining.

The karyotype represented here is slightly different from that of the previous report by Yuasa & Maekawa (1976). They reported that *H. okinawensis* contained one pair of small subtelocentric chromosomes instead of small subtelocentric SAT-chromosomes. In this study, however, I confirmed that this species also possessed one pair of the small subtelocentric SAT-chromosomes in a chromosomal complement.

H. sakawana, H. costata and *H. minamitania.* These three closely related species had the same chromosome number 2n=24 (Fig. 1E, F, G), and there was no difference in karyotype and C-banding pattern among the three species (Fig. 2E, F, G; Pl. IE, F, G). Therefore, detailed description of karyotype and C-banding pattern is to be made on *H. sakawana.* In the karyogram of this species was found one pair of small subtelocentric chromosomes which, unlike those of *H. aspera* and *H. okinawensis* stated above, exhibited the C-band only at the centromeric region (see Fig. 3B). It was a characteristic chromosome in a complement of this species. The other chromosomes were metacentric. The metacentric pairs no. 1 and no. 2 were slightly longer than the rest, and occasionally showed secondary constrictions on both arms. All the metacentric chromosomes

showed centromeric C-bands which appeared either as a single band or as two dots. The broad centromeric C-band was often observed in two middle-sized metacentric chromosomes. In Giemsa C-stained interphase nucleus were found many chromocenters which were slightly different in size (Pl. IH).

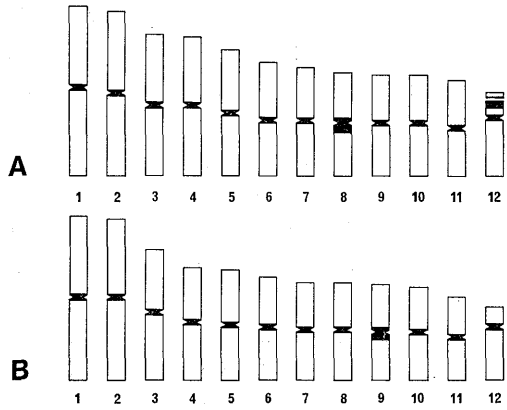

Fig. 3. Idiograms showing the haploid set of the C-banded metaphase chromosomes. A: *Heterotropa aspera*. B: *Heterotropa sakawana*.

Discussion Among the seven species here examined no great differences exist not only in chromosome number and karyotype but also in C-banding pattern. However, conspicuous karyological differentiations, especially with reference to the small chromosomes, are recognized among these species (Fig. 3).

In four species, *H. aspera*, *H. hexaloba* var. *perfecta*, *H. subglobosa* and *H. okinawensis*, one pair of small subtelocentric SAT-chromosomes is commonly found in each chromosomal complement, which shows C-bands both at the centromeric and at the distal regions, but a satellite region is negative to Giemsa C-staining. The chromosomal pair of this type is in good agreement with those found in seven species previously reported (Sugawara 1981).

In contrast, the remaining three species, *H. sakawana*, *H. costata*, and *H. minamitania*, have no subtelocentric SAT-chromosomes in each chromosomal complement. This is the most noteworthy result of the present study as discussed later. In these three species is there one pair of small subtelocentric chromosomes in common in each chromosomal complement, and it possesses C-band only at the centromeric region (Fig. 3B). Thus, the subtelocentric chromosome of this type is clearly discriminated from the subtelocentric SAT-chromosome stated above.

As compared with the latter chromosome, the subtelocentric chromosome is slightly shorter in the length of the short arm and lacks a heterochromatic segment on the distal region of the short arm. In addition, as far as the karyotypes and

C-banding patterns are known within the genus *Heterotropa*, the presence of one pair of the small subtelocentric chromosomes without satellite is restricted to these three species. It is therefore considered that the subtelocentric chromosome may have derived from the subtelocentric SAT-chromosome by deficiency of the distal region of the short arm. To confirm the above-mentioned point, however, more detailed cytological investigations are needed.

According to Araki (1937, 1953), it was considered that the three species of *H. sakawana*, *H. costata* and *H. minamitania* had a close relationship to the two species of *H. aspera* and *H. hexaloba*, and these five taxa were grouped into the same section (sect. Aschidasarum) together. While, Maekawa (1933, 1972) suggested that the former three species should be assigned to a distinct section (sect. Sakawanae) separated from the latter two taxa, since the former ones were easily distinguished from the latter by having a calyx tube with longitudinal lamellae on the inner surface. As already stated above, the three species of *H. sakawana*, *H. costata* and *H. minamitania* are clearly different from *H. aspera* and *H. hexaloba* var. *perfecta* in form and C-banding style of the shortest chromosome in chromosomal complement. Therefore, the three species of *H. sakawana*, *H. costata* and *H. minamitania* may be a distinct group also from cytotaxonomic viewpoint, which are characterized by having one pair of the small subtelocentric chromosomes in each chromosomal complement.

H. okinawensis is an endemic species restricted to Okinawa Island. Previously, Yuasa & Maekawa (1976) suggested that the species *H. okinawensis* may represent an ancestral condition in karyotype within the genus *Heterotropa*, since this species had no subtelocentric SAT-chromosomes in a chromosomal complement and grew in the Paleozoic lime-stone area in the Ryukyu Islands. However, no support for the above suggestion is available from the information obtained here, because *H. okinawensis* also contains one pair of small subtelocentric SAT-chromosomes in its karyotype which is common to all of the species concerned, except for a few species: *H. sakawana*, *H. costata* and *H. minamitania*.

In conclusion, the present study clearly shows that the three species of *H. sakawana*, *H. costata* and *H. minamitania* are unique among the species of *Heterotropa* in karyotype and C-banding pattern, especially of one pair of small chromosomes.

I am grateful to Prof. M. Ono of Tokyo Metropolitan University, for his kind suggestion and encouragement during the course of this work, and also for his critical reading of the manuscript.

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Explanation of Plate I

Plate I. C-bands in metaphase and interphase nucleus. A, B. *H. aspera*. C. *H. subglobosa*. D. *H. okinawensis*. In A-D, arrows indicate the small subtelocentric SAT-chromsomes in which the C-bands appear at the centromeric and at the distal regions of the short arm, the satellite being negative to Giemsa stainining. E. *H. sakawana*. F, G. *H. costata*. In E-G, arrows indicate the small subtelocentric chromosomes in which the C-band appears only at the centromeric region. H. Giemsa C-banded interphase nucleus in *H. sakawana*. All: ×1500.

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九州，四国地方に生育するカンアオイ属 *Heterotropa* 7 種の核型及び C-バンド分染型を分析したのでその結果を報告した。先の菅原 (1981) の報告では，カンアオイ属の多くは 2n=24 の染色体数をもち，核型はほとんど中部型染色体から構成され，最小の一

対のみが次端部型染色体で短腕に付随体をもつということが述べられた。ところが，このたび観察したサカワサイシン（*H. sakawana*），トサノアオイ（*H. costata*），オナガカンアオイ（*H. minamitania*）の三種では，そのような付随体をもつ次端部型染色体が全く認められず，最小の染色体は次端部型染色体でおきかえられていた。このような染色体の存在は，この3種においてのみみられるものであった。次端部型染色体は，付随体をもつ次端部型染色体と比較した場合，その短腕部がいくぶん短く，異質染色質を欠くことなどから，末端部の欠失によって二次的に派生したものと考えられる。また分類学的に，これら3種を含むサカワサイシン節（sect. Sakawanae）とミヤコアオイ節（sect. Aschidasarum）とを同一節にまとめるべきという意見もあるが，これら二つの節の間には核型，C-分染型のうえでの大きな違いがみられ，それを考慮すると，むしろそれぞれ独立した節として取り扱うのが妥当と思われる。

□環境庁自然保護局：**屋久島原生自然環境保全地域調査報告書** 714pp. 1984. 同局，非売品. 日本自然保護協会が調査を担当し，植物分類学関係では光田重幸・永益英敏氏（京大）による維管束植物の全既知種の目録（文献調査）と調査地域での採集植物目録，平野 実・光田重幸氏による淡水珪藻・鼓藻の採集品目録，土永浩史氏（奈良教育大）の蘚苔類目録がある。植物生態学関係では6篇の調査報告がある。このほか気候・地質・地形・土壌，動物関係の調査報告が含まれている。（金井弘夫）

□巌佐耕三・前田みね子・斎藤捷一：**生物生存の原理を探る　プロチスタ生物学入門** 227 pp. 1984. 培風館，東京. ¥2,100. プロチスタ（著者等によると動物でも植物でもない生物）について書いた本である。といってもプロチスタに属する細菌，粘菌，真菌，藻類，原生動物等についての解説本ではなく，多様な構造と機能をもち，多様な生きざまを示すプロチスタに視点をおいた，生物に共通する基本法則を理解する本である，と著者等は述べている。内容はプロチスタとは何か，体制，分化と造形，増殖と生殖，運動，走性，生態系，個体群，種と進化などの章から成る。各論的でないとは言え，微細藻と粘菌の専門家による本であり，プロチスタについて豊富な情報が盛られ，プロチスタ自体の理解にも大いに役立つ。大学の教養教育を永年担当された経験と著者等を中心に数年続けてきたというプロチスタ・ゼミの成果が生かされ，専門的であるが理解し易い内容の本となっている。欲を言えば，図と表に一工夫ありたいところ。さらに親しみ易くなったであろう。（千原光雄）

○三宅島の降石原に見出されたゼニゴケとヒョウタンゴケ（大場秀章）Hideaki OHBA: Occurrence of two bryophytes on a eight-month-old scoria field in Miyakejima Island, Tokyo

伊豆諸島三宅島では 1643, 1712, 1834, 1874, 1940, 1962年と噴火を繰り返してきたが，再び1983年10月３日に噴火が起った。東京大学が行った火山島に関する特定研究の１員として噴火による植物相の変化を調べるため継続して渡島する機会が与えられた。

三宅島の植生は流出した溶岩，降石（スコリア），降灰，地熱上昇などの影響を受けた。噴火から８ヶ月過ぎた1984年6月でも，依然火口周囲では地熱が高く，数ヶ所で水蒸気が立ち昇り続けている。流出した溶岩の上にはまだ可視的な植物はみられない。かつてうっそうとした森林に被われていた新澪池西側は，多量のスコリアの堆積とその激しいボンビング(bombing)や地熱の急上昇による地下部の焼焦などのためもとの植生は一変した。少くとも林床植物はすべて枯死した。スダジイ，タブ，エノキ，オオバヤシャブシ，ヒメユズリハ，ハチジョウイボタなどからなる森林を構成していた樹木もボンビングを受けほとんどが枯れたが，ハチジョウイボタ，タブ，スダジイ，エノキの少数の個体が幹生枝を出し新しい葉を展開していた。

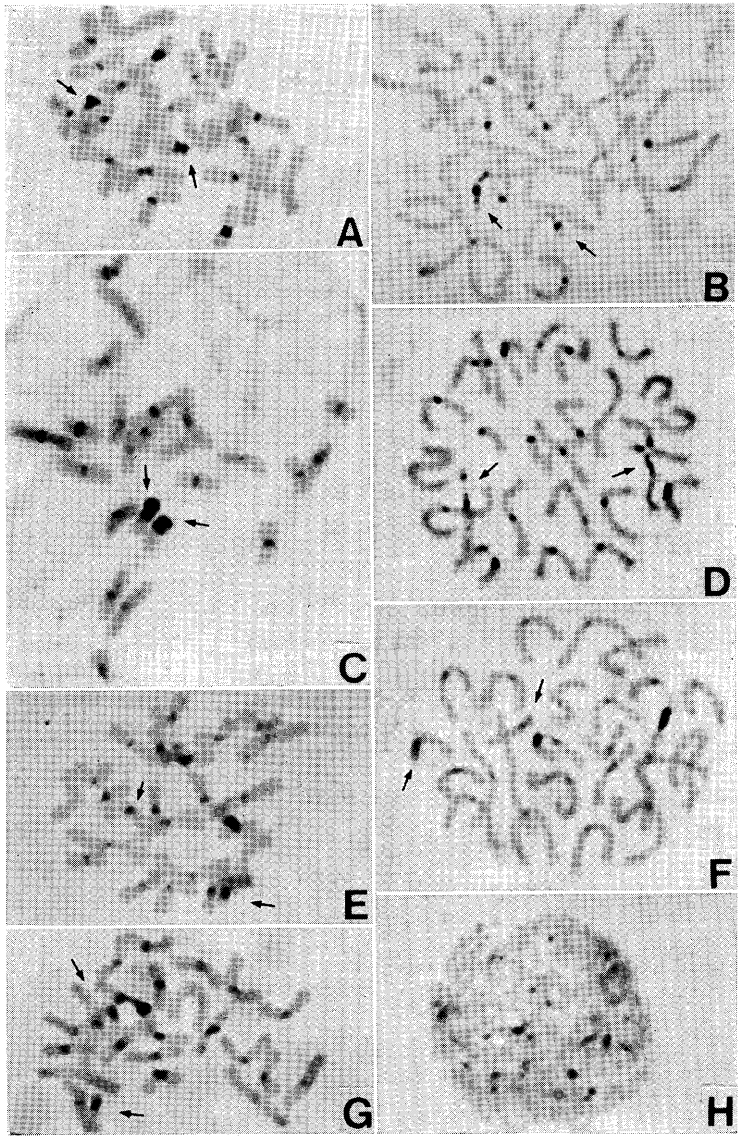
この降石原には噴火後１ヶ月に *Pyronema omphalodes* (Bull. ex Fr.) Fuckel（菌類）が発生し採集されている。３ヶ月後には *Caloplaca* sp.（地衣類）が多量に発生した。*Caloplaca* は地表からわずかに突出した大形の放出岩塊の，主に東向きの地表面との境界に沿ってコロニーを形成していた。これはここで卓越する風の向きに一致している。海風が塩類を運び岩面に付着させ，その塩類が保水に役立っていると考えられる。

さて，1984年６月には数種の菌類のほかに，広分布をする２種のコケ植物，ゼニゴケ (*Marchantia polymorpha* L.) とヒョウタンゴケ (*Funaria hygrometrica* Hedw.) が見出された。２種とも1984年４月頃から出現していたようである。６月に調べた時はヒョウタンゴケは蒴を多数つけていた。ゼニゴケは *Caloplaca* のコロニー上に生え，雌雄両株ともみられ，ともに生殖器を有していた。ヒョウタンゴケはたき火のあとに好んで生えるとされ，ゼニゴケもヨーロッパでは山火事の跡などを好んで生える傾向があるという。この２種のコケが裸地化を受けた植生の初期に出現したことと環境との関係については判っていない。

噴火直後の裸地にどのような植物が生えるのか植物学的に興味深い問題であるが，日本ではこの種の記録は少ないように思われる。三宅島のこの場所で今後どのような種が出現し，または消滅してゆくのか継続して観察を続けて行きたいと思っている。

同定や種々の御助言を賜った井上　浩（科博），柏谷博之（科博），北川尚史（奈良教育大），土居祥允（科博），浜田隆士（東大），綿秩邦彦（東大）博士に感謝の意を表します。

（東京大学 総合研究資料館）



T. SUGAWARA: Karyotype of *Heterotropa*