

ISSN 0386-5304

No.30 Mar.2012

**Bulletin of**  
**The Hiroshima Botanical Garden**

**Published by**

The Hiroshima Botanical Garden  
(Municipal)  
Kurashige, Sacki-ku, Hiroshima  
Japan

## CONTENTS

- Kenzo Hitotsubashi, Yoshiko Kondo, Takato Saito, Naoko Inoue, Tetsuya Sera : Memoranda for the Flora of Hiroshima Prefecture(6) Notes on the species of *Carex* (Cyperaceae), recorded in Hiroshima Prefecture ..... 1 - 6
- Tetsuya Sera, Tomoyo Nishida, Genjiro Ishida and Yoshikazu Hoshi : A comparative population study of genetic diversity in *Goodyera foliosa* var. *laevis* (Orchidaceae) native to Hiroshima Prefecture, Japan ..... 7 - 14
- Shuichi Hamatani and Mikio Aoyama : Karyomorphological studies of six species of subtribe Catasetinae, Orchidaceae ..... 15 - 30
- Tetsuya Sera, Mikio Aoyama and Genjiro Ishida : Karyomorphological observation on 14 species of subtribe Stanhopeinae, Orchidaceae ..... 31 - 50

## 目次

- 一橋賢三・近藤芳子・斉藤隆登・井上尚子・世羅徹哉：広島県フロラ覚書(6)  
広島県に自生するスゲ属植物に関する新知見 ..... 1 - 6
- 世羅徹哉，西田知世，石田源次郎，星良和：広島県に自生するアケボノシユスランを用いた  
RAPD 解析 ..... 7 - 14
- 濱谷修一・青山幹男：カタセタム亜族（ラン科）6種の核形態学的研究 ..... 15 - 30
- 世羅徹哉・青山幹男・石田源次郎：ラン科スタンホペア亜族14種における  
染色体観察 ..... 31 - 50



## 広島県フロラ覚書(6) 広島県に自生するスゲ属植物に関する新知見\*

一橋賢三<sup>1)</sup>・近藤芳子<sup>2)</sup>・斉藤隆登<sup>3)</sup>・井上尚子<sup>4)</sup>・世羅徹哉<sup>4)</sup>

日本産のカヤツリグサ科スゲ属には200種以上が記載されており、高等植物の中では属内の種数が最も多く、乾燥地から湿地、日向から陰地まで生態的に様々な場所に生育している(星野ほか2002)。広島県内のスゲ属植物の分布を調べることは、広島県をより深く理解することにつながると期待される。

広島県に自生するスゲ属植物については、高木哲夫が1945年までに75種、4亜種、20変種、4品種(広島市植物公園(編)2005)、Okamoto(1965)が72種5変種、土井義夫(1983)が67種13変種を報告し、江塚・松本(1985)が福山市周辺で52種6変種を報告している。その後も県内の自生植物について解明が進み、87種12変種、1品種のスゲ属植物の自生が確認されている(広島県植物誌1997、広島県植物誌補遺2010)。ところが、2010年以降も広島県新産種や希少種の新産地が発見されるなどの新知見があったので、ここにまとめて報告する。

新知見の記載は、以下の要領で行った。

1. 星野・正木・西本(2011)の配列に従って、種ごとに列記した。
2. 学名は星野・正木・西本(2011)に従ったが、一部は勝山(2005)を引用した。
3. 引用した文献のうち、次の3点は「略称」で記した。①広島市植物公園(編)2005:「高木リスト」、②広島大学理学部附属宮島自然実験所・比婆科学教育振興会(編)1997:「広島県植物誌」、③世羅徹哉・坪田博美・松井健一・浜田展也・吉野由紀夫2010:「植物誌補遺」。
4. 各種の記述は、学名、和名、「逸出」等の情報、備考、引用文献、産地と引用標本、<>内に、広島県植物誌と植物誌補遺に記載された産地、という順序で行った。尚、標本のhbgは広島市

植物公園に、HIRO-MYは広島大学大学院理学研究科附属宮島自然植物実験所に、OKAYは岡山理科大学に保管してあることを示す。

### Sect. *Grallatoriae* ヒナスゲ節

#### *Carex grallatoria* Maxim. ヒナスゲ

広島市佐伯区の自生地は、標高1050mの山の北斜面で、海拔950mあたりから山頂付近まで分布し、樹林下の巨大な岩上に、マット状に生育していた。岩の上面には腐植がたまり、本種のほかにヤマシグレ、ダイセンミツバツツジ、コックバネウツギ、ツクバネソウ、ヤマグルマ等の生育が認められた。生育地は、ミズナラ、クリ、コハウチワカエデなどからなる落葉広葉樹林であった。今回の自生地はすでにOkamoto(1965)が報告しているが、広島県植物誌編集時には標本が確認されなかったため掲載されなかった。著者らは2011年5月20日に雄株、雌株の生育を確認した。

(文献)高木リスト, Okamoto(1965), 正木(2011)  
(標本)広島市佐伯区湯来町(hbg-20257, hbg-20271; 星野卓二 同定)

### Sect. *Rarae* ハリスゲ節

#### *Carex fulta* Franch. ニッコウハリスゲ

ブナ帯の湿地や流水域に生える多年草。Okamoto(1965)が庄原市口和町の自生を報告しているが、広島県植物誌編集時には標本が確認されなかったため掲載されなかった。今回報告する自生地の海拔高度は550mで、上部中間温帯に属す(鈴木ほか1979)。自生地は林縁にある流水沿いの湿潤地で、近くにはサクラソウやホソバナアマナ、タマツリスゲ、コジュズスゲなども生育していた。

(文献)Okamoto(1965)  
(標本)庄原市東城町(hbg-20226, hbg-20234; 星野卓二 同定)

\*Contribution from the Hiroshima Botanical Garden No.94

1) 広島市植物公園ガイドボランティア, 2) 広島市安佐南区長東, 3) 広島市立東野小学校, 4) 広島市植物公園 Bulletin of the Hiroshima Botanical Garden, No.30: 1-6, 2012

**Carex capillacea** Boott ハリガネスゲ

広島県内に広く分布することが知られていたが、広島県植物誌編集時には標本が確認されなかったのが掲載されなかった。

(文献) 高木リスト, 土井 (1983), 江塚 (1994), すげの会 (編) (2009b)

(標本) 廿日市市吉和 (hbg-15118), 廿日市市佐伯町 (hbg-18790), 安芸太田町戸河内 (hbg-18912), 東広島市豊栄 (hbg-19899), 広島市安佐南区 (hbg-19977), 庄原市東城町 (hbg-20132; 星野卓二 同定), 北広島町芸北 (hbg-20106; 星野卓二 同定)

**Sect. Phleoideae** ミノボロスゲ節**Carex nubigena** D. Don ex Tilloch et Taylor subsp.**Albata** (Boott ex Franch.) T. Koyama ミノボロスゲ

広島県植物誌では未確認種として掲載され、星野ほか (2011) では、分布域は岡山県以东となっている。著者の一人、斉藤が広島県内で自生を確認したが、スキー場の周辺の吹き付け植栽周辺であったことから、逸出の可能性がある。

(文献) 高木リスト

(標本) 北広島町芸北 (hbg-20110; 星野卓二 同定)

**Sect. Vulpinae** オオカワズスゲ節**Carex stipata** Muhl. ex Willd. オオカワズスゲ

戦前の記録があるが、これまでの調査では標本が確認されていない。2002年頃、佐藤克則によって確認された。本種は、東ロシアや北アメリカにも分布し、日本では北海道、本州の中部地方以北に分布の中心があり、鳥取県と広島県に隔離分布する (星野ほか 2011)。発見当初、自生地が空港に隣接していることや、周辺の法面には移入とされる「イワヨモギ」の生育が見られたことなどから、人為的な要因で入ってきた可能性が考えられた (佐藤克則 私信)。今回の調査で、自生地の周辺にジュンサイ、ガマ、ヤチカワズスゲ、コシロネ、タチスゲ、アブラガヤ、サクラバハンノキなど湿地性の植物を確認したが、オオカワズスゲが自生か逸出かの判断はできなかった。

(文献) 高木リスト, すげの会 (2010), 星野ほか (2011)

(標本) 東広島市河内町 (hbg-20243; 星野卓二 同定), 三原市本郷町 (OKAY-21835)

**Sect. Remotae** ヤブスゲ節**Carex planata** Franch. et Sav. var. *planata* タカネマスクサ

北海道~九州に分布し、平地から山地に生育するところがある (勝山 2005)、広島県内ではこれまで、中~東部にしか自生が知られていなかった。今回の標本採集地は、広島市の北西部で、広島県西部に位置する。この生育地は舗装されていない林道沿いの少量の流水が見られる周辺で、ミゾソバやイボクサ、アシボソ、アキノウナギツカミ等と共に生えていた。付近には、アラカシ、リンボク、ヤブツバキなど常緑広葉樹が見られた。

(文献) 高木リスト, Okamoto (1965), 土井 (1983), 江塚・松本 (1985), 広島県植物誌, 植物誌補遺, すげの会 (編) (2010), 正木 (2011)

(標本) 広島市安佐北区瀬谷 (hbg-20184; 星野卓二 同定) <西城町, 吉田町, 帝釈峡, 総領町>

**Sect. Graciles** ナキリスゲ節**Carex autumnalis** Ohwi オオナキリスゲ

日本では本州の近畿地方以西、四国の限られた地域に分布し、国外では中国に記録がある (星野ほか 2011)。広島県ではこれまで県東部でのみ自生が知られていたが、今回、広島市の南部や呉市の自生が確認された。

(文献) 高木リスト, Okamoto (1965), 土井 (1983), 植物誌補遺

(標本) 呉市 (hbg-19274), 広島市南区 (hbg-19496) <神石町, 福山市>

**Sect. Phacocystis** アゼスゲ節**Carex maximowiczii** Miq. var. *levisaccus* Ohwi ホシナシゴウソ (広島県新記録)

ゴウソの集団に混じって見られるが、個体数は少なく、分布域も限られる (星野ほか 2002)。これまで広島県内の自生の記録がないが、ゴウソと区別されていない可能性も考えられる。

(標本) 廿日市市佐伯町 (hbg-20070), 廿日市市極楽寺山 (hbg-20064)

**Sect. Mundae** ミヤマジュズスゲ節**Carex dissitiflora** Franch. ミヤマジュズスゲ

Okamoto (1965) らによって知られていたが、広島県植物誌編集時には標本が確認されなかったのが掲載されなかった。

(文献) Okamoto (1965), 江塚・松本 (1985)

(標本) 庄原市東城町 (hbg-20142; 星野卓二 同定), 廿日市市吉和 (hbg-20160; 星野卓二 同定)

**Sect. Rhomboidales ヒエスゲ節*****Carex laticeps* C. B. Clarke ex Franch. オオムギスゲ**

国内では本州（愛知県、瀬戸内海沿岸）、四国（小豆島）の、日当たりの良いやや乾燥した場所にまれに見られ、国外では朝鮮半島、中国に分布する（星野ほか 2011）。この分布型は広島県の南東部から岡山県の南西部を中心とした限られた地域にしか分布せず、国外では朝鮮半島、中国に分布するという点で、前川（1949）がいう「阿哲要素」の植物の特徴と一致する。岡山理科大学の星野研究室ではその系統地理学的研究に取り組んでいて（星野 私信）、その謎が明らかにされることが期待される。

（文献）高木リスト、土井（1983）、江塚・松本（1985）、植物誌補遺

（標本）尾道市因島（hbg-19871）＜福山市、三原市＞

***Carex papillaticulmis* Ohwi アオバスケ**

広島県内でこれまでに確認されている自生地は広島市の東郷山と大峯山（未発表）で、それぞれ海拔800mと920mであり、上部中間温帯もしくはブナ林域（広島県植物誌）に位置した。今回の調査地は海拔高度520mで、下部中間温帯に属す場所であった（鈴木ほか 1979）。

（文献）広島県植物誌

（標本）廿日市市佐伯町（hbg-20165；星野卓二 同定）  
＜東郷山＞

**Sect. Mitratae ヌカスゲ節*****Carex* sp. ウスイロヒメカンスゲ（広島県新記録）**

瀬戸内海の島嶼部や沿岸地域に広く分布する。ヒメカンスゲとは葉が明るい緑色で、苞の鞘は淡緑色、雄鱗片と雌鱗片の先端は凹状であること、雌鱗片は淡緑色、染色体数は $2n=32$ であることなどで区別される（星野ほか 2011）。この分類群は未記載で、これまで広島県植物誌ではヒメカンスゲの人里型、勝山（2005）ではヒメカンスゲの瀬戸内タイプと呼ばれていたものである。

（標本）福山市鞆（hbg-19800）、福山市山野（hbg-16360）、広島市佐伯区（hbg-16327）、世羅町（hbg-19917）。3点とも星野卓二 同定。

***Carex multifolia* Ohwi var. *pallidisquama* Ohwi アオミヤマカンスゲ（広島県新記録）**

山地のやや湿った林床に生える。ミヤマカンスゲ

の染色体数は $2n=30, 60, 62, 64-66, 70$ だが、アオミヤマカンスゲは $2n=72$ で、ミヤマカンスゲとは鱗片が淡緑色、基部の鞘が淡褐色であることなどで区別される（星野ほか 2011）。これまで広島県内でミヤマカンスゲと同定していたものに、アオミヤマカンスゲが含まれている可能性がある。

（標本）福山市（hbg-16349；星野卓二 同定）、広島市佐伯区（hbg-19786）、世羅町（hbg-19917）

***Carex alterniflora* Franch. var. *fulva* Ohwi キイトスゲ**

山地あるいは山頂付近に生える。本種の分布域は、本州中部以北とされていたが、最近岡山県、鳥取県、兵庫県、愛媛県で採集され、西日本にも広く分布していることが明らかになった（星野ほか 2011）。県内では2003年に初めて確認されたが（斉藤 2003）、その後も新たな産地が確認されたので、記録する。自生地はブナ帯で、中国山地の尾根または山頂部である。

（文献）斉藤（2003）、植物誌補遺

（標本）安芸太田町（hbg-20238）、北広島町深山（hbg-14811）。2点とも星野卓二 同定 ＜芸北町＞

***Carex alterniflora* Franch. var. *arimaensis* Ohwi アリマイトスゲ**

基準産地は兵庫県の有馬で、近畿地方と岡山県で自生が知られていた（星野ほか 2002）。その後鳥取県（正木 2004）、愛媛県（得居 2006）と新産地が発見され、2009年に著者の一人、斉藤が広島県の自生を確認した。広島県の自生地は、県西部の道端のやや乾燥した場所である。

（文献）すげの会（編）（2009c）

（標本）北広島町豊平（hbg-17698；星野卓二 同定）、広島市安佐北区（hbg-19978）

***Carex pudica* Honda マメスゲ**

本州（宮城県以西）、伊豆諸島（神津島）に分布する日本固有の植物で、湿原周辺や草地に生える（星野ほか 2011）。同定の決め手となる有花茎が葉に隠れて見えにくいためと思われるが、広島県内の産地情報は少ない。

（文献）植物誌補遺

（標本）安芸高田市甲田（hbg-20121；星野卓二 同定）  
＜芸北町＞

***Carex puberula* Boott イトアオスケ（広島県新記録）**

広島県植物誌はイトアオスゲ (*Carex breviculmis* R.Br. var. *discoidea* (Boott) Boott) を掲載している。これは大井 (1978) に従ったもので、勝山 (2005) や星野ほか (2011) はこれをヒメアオスゲ *Carex discoidea* Boott とし、*Carex puberula* Boott をイトアオスゲの名で別の分類群に扱っている。2011年に北広島町で採集された個体が *Carex puberula* であった (hbg-20118; 星野卓二 同定)。

一方 *Carex discoidea* Boott (ヒメアオスゲ) は、海岸近くの草地や日当たりの良い林床に生えるとされ (星野ほか 2011)、広島県内で記録されている個体も宮島の海岸近くに生えたものであった (HIRO-MY-886, HIRO-MY-25800, HIRO-MY-32065 関太郎 同定)。しかし、今回、広島市佐伯区の海岸から約 10km 離れた山地 (海拔 500m) でもヒメアオスゲの自生を確認した (hbg-19881; 星野卓二 同定)。

#### Sect. *Acrocystis* ヒメスゲ節

##### *Carex mira* Kük. サワヒメスゲ

川岸の岩上に生えると言われ (勝山 2005, 星野ほか 2011)、これまで広島県で知られていた自生地も太田川流域の川岸の岩上であったが、今回、準平原地域のため池の土手に群生しているのを確認した。

(文献) 高木リスト, Okamoto (1965), 土井 (1983), 広島県植物誌, 植物誌補遺  
(標本) 三次市三和町 (hbg-20126; 星野卓二 同定)  
<広島市南原峡, 加計町>

#### Sect. *Molliculae* ヒメシラスゲ節

##### *Carex pseudoaphanolepis* Ohwi アイノコシラスゲ (広島県新記録)

シラスゲの中には葉があまり粉白でなく、雌小穂が垂れ下がらない型がある。これは、シラスゲとエナシヒゴクサの雑種として上記学名で記載された型に当たるが、星野ほか (2011) はこの型を載録していない。勝山 (2005) はこの型を雑種ではないとしたうえで独立した分類群に相当するかどうかは今後の研究課題だとしている。広島市南区で採集された個体は、アイノコシラスゲと呼ばれる型であったので、区別して記録する。

(標本) 広島市南区 (hbg-20241; 星野卓二 同定)

#### Sect. *Rostraloës* ミタケスゲ節

##### *Carex dolichocarpa* C. A. Mey. ex Kom. ミタケスゲ (広

##### 島県新記録)

湿原に生える。日本国内ではこれまで中部以北で知られていたが、最近、岡山県北部や九州 (大分県) にも隔離分布することが明らかになった (星野ほか 2011)。著者の一人近藤が、2009年に広島県中央部の吉備高原面の湿地に本種と思われる植物が生育していることに気づき、後に星野卓二教授が確認したものである。周辺にヌマガヤ、ヤチカワズスゲ、マメスゲ、モウセンゴケ、ミミカキグサ、トキソウ、サギソウ、コタヌキモ、オモダカ、オオミズゴケ、サクラバハノキ、レンゲツツジなど湿地性の植物が見られた。これまでに6ヶ所の湿地で生育を確認したが、そのうち一ヶ所では、2011年に生育が認められなかった。また、最大の湿地は周辺の森林が伐採されており、今後、環境の変化による悪影響が心配される状態である。

(標本) 東広島市 (hbg-18553), 三次市三和町 (hbg-19886), 安芸高田市甲田町 (hbg-20249; 星野卓二 同定)

#### Sect. *Paludosae* シオクグ節

##### *Carex pumila* Thunb. コウボウシバ

海岸砂浜に生える。本種の県内の産地情報は、高木リストでは宮島、生野島、己斐、忠海、仙酔島、他の2地点で合計7地点、土井 (1983) には、宮島、廿日市、五日市、井口、府中町の5地点あったのに対し、広島県植物誌には倉橋島と走島の2地点で、宮島の自生が絶滅したことも報告していて、自生環境が悪化したことが推測される。

(文献) 高木リスト, Okamoto (1965), 土井 (1983), 江塚・松本 (1985), 広島県植物誌  
(標本) 三原市 (hbg-20054) <宮島 (絶滅), 倉橋島, 福山市走島>

#### Sect. *Carex* ビロードスゲ節

##### *Carex miyabei* Franch. ビロードスゲ

砂質の湿草地、山道脇に生える (星野ほか 2011)。Okamoto (1965) が報告していた北広島町大朝の自生地周辺では、2011年の調査で、自生を確認できなかった。これは護岸工事や、ツルヨシなど大型の湿生植物の侵入によってビロードスゲに適した環境が減少したためと思われる。

(文献) Okamoto (1965), 江塚・松本 (1985), 広島県植物誌  
(標本) 北広島町芸北 (hbg-19998; 星野卓二 同定)

<大朝町大塚, 帝釈峽>

## 謝 辞

本稿をまとめるにあたり, 岡山理科大学の星野卓二教授と正木智美先生には標本同定と情報提供をしていただきました。東和環境科学株式会社の吉野由紀夫氏, 中外テクノス株式会社の佐藤克則氏には情報提供をしていただきました。広島大学大学院理学研究科附属宮島自然植物実験所の坪田博美准教授, 向井誠二氏, 向井美枝子氏には標本閲覧の便宜を図っていただきました。広島市植物公園ガイドボランティアの北本照子氏には自生地調査の協力を得ました。お名前を記して感謝の意を表します。

## 引用文献

- 土井美夫 1983. 広島県植物目録. 148pp. 博新館, 広島県.
- 江塚昭典 1994. 農業環境技術研究所所蔵植物標本目録 - 1993年現在 -. 農業環境技術研究所資料 15: 1-153.
- 江塚昭典・松本和夫 1985. 福山市周辺の植物相. 中国農事試験場報告 E23: 1-107.
- 広島大学理学部附属宮島自然植物実験所・比婆科学教育振興会(編) 1997. 広島県植物誌. 832pp. 中国新聞社, 広島県.
- 広島市植物公園(編) 2005. 高木リスト 広島県産高等植物目録. 広島市植物公園紀要 22-23: 5-129.
- 星野卓二・正木智美・西本眞理子 2002. 岡山県スゲ属植物図譜. 229pp. 山陽新聞社, 岡山県.
- 星野卓二・正木智美・西本眞理子 2011. 日本カヤツリグサ科植物図譜. 781pp. (株)平凡社, 東京.
- 勝山輝男 2005. ネイチャーガイド 日本のスゲ. 376pp. (株)文一総合出版, 東京.
- 前川文夫 1949. 日本植物区系の基礎としてのマキネシア. 植物研究雑誌 24:91-96.
- 正木智美 2004. アリマイトスゲの新産地. すげの会ニュース 2.
- 正木智美 2009. すげの会ニュース 20: 19.
- 正木智美 2011. 広島県内新記録など<緊急ニュース>. すげの会ニュース 23.
- Okamoto, K. 1965. Taxonomic study of the Carices in the western Honsyu of Japan. Bull. Okayama Coll. Sci. 1: 1-105.
- 大井次三郎 1978. 改訂増補新版 日本植物誌 顕花篇. 1, 585pp. 至文堂, 東京.
- 斉藤隆登 2003. 芸北町産スゲ属植物(1) キイトスゲ. 刈尾(西中国山地自然史研究会会報) 12: 7.
- 世羅徹哉・坪田博美・松井健一・浜田展他・吉野由紀夫 2010. 広島県植物誌補遺. 広島市植物公園紀要 28: 1-74.
- すげの会(編) 2009a. 日本産スゲ属植物分布図集(試案) no. 1. 33pp.
- すげの会(編) 2009b. 日本産スゲ属植物分布図集(試案) no. 2. 50pp.
- すげの会(編) 2010. 日本産スゲ属植物分布図集(試案) no. 3. 86pp.
- すげの会(編) 2011. 日本産スゲ属植物分布図集(試案) no. 4. 105pp.
- 鈴木兵二・豊原源太郎・安藤久次・中野武登 1979. 広島県の植生図解説書. 72pp. + 2付図. 広島県. 得居 修 2006. アリマイトスゲが愛媛県にも分布する. すげの会ニュース 8.

## 摘 要

1. 本調査で, ミタケスゲ, ホシナシゴウソ, ウスイロヒメカンスゲ, アオミヤマカンスゲ, イトアオスゲ, アイノコシラスゲの6分類群が, 広島県で初めて記録された。
2. 広島県植物誌補遺(2010)以降, 現地調査, 証拠標本調査, 文献調査等によって, 新たにスゲ属植物 13 分類群の広島県内の自生を確認した。
3. 広島県のスゲ属 9 種について, 新たな分布情報を追加した。



**Memoranda for the Flora of Hiroshima Prefecture(6)**  
**Notes on the species of *Carex* (Cyperaceae), recorded in Hiroshima Prefecture**

Kenzo Hitotsubashi <sup>1)</sup>, Yoshiko Kondo <sup>2)</sup>, Takato Saito <sup>3)</sup>, Naoko Inoue <sup>4)</sup> and Tetsuya Sera <sup>4)</sup>

**Summary**

1. Distribution of six taxa of *Carex*; *C. dolichocarpa*, *C. maximowiczii* var. *levisaccus*, *C. sp.* (Japanese name 'Usuiro-himekansuge'), *C. multifolia* var. *pallidisquama*, *C. puberula* and *C. pseudoaphanolepis* are reported from Hiroshima Prefecture for the first time in this study.
  2. Based on previous literature, herbarium specimens and our investigations since 2010, an additional 13 *Carex* plant taxa, including infraspecific taxa, are confirmed for Hiroshima Prefecture.
  3. Additional informations about the distribution of 9 species of *Carex* plants in Hiroshima are given.
- 

1) Volunteer guide staff of the Hiroshima Botanical Garden

2) Nagatsuka, Asaminami-ku, Hiroshima City

3) Higashino Elementary School, Hiroshima City

4) The Hiroshima Botanical Garden

## A comparative population study of genetic diversity in *Goodyera foliosa* var. *laevis* (Orchidaceae) native to Hiroshima Prefecture, Japan\*

Tetsuya Sera<sup>1)</sup>, Tomoyo Nishida<sup>2)</sup>, Genjiro Ishida<sup>1)</sup> and Yoshikazu Hoshi<sup>3)</sup>

### Abstract

Molecular analysis was carried out using random primers to infer the characteristics of RAPD generated DNA fragments and to investigate genetic diversity of six populations of *Goodyera foliosa* var. *laevis* in Hiroshima Prefecture, Japan. Using two decamer primers of OPA-01 and OPB-05, 157 reproducible DNA fragments were obtained from 30 individuals of this variety. In cluster analysis, four main clades were found in the tree. One of these clades consisted of all five individuals of Mt. Tateeboshiyama population.

### Introduction

*Goodyera* R. Br. is a member of the Orchidaceae and comprises 40 species widely distributed from the subarctic regions of the Northern Hemisphere to tropical regions in Asia (Satomi 1982). All species in this genus are terrestrial, with one exception. *Goodyera* species mainly grow in primary forests with high humidity and are often associated with dominant forest-trees. Thus, it is suggested that speciation of this group is correlated with certain associated forest-trees (Tanaka 1965).

Taxonomic treatments of *Goodyera*, however, are still imperfect, because the genus contains many rare species with not enough complete herbarium specimen masses (Schlechter 1926, Holttum 1964, Briger 1974-1975, Dressler 1981). Therefore, more examinations must be necessary to clarify these taxonomic treatments, as pointed out by some previous works (Dressler and Dodson 1960, Maekawa 1971, 1978, Seidenfaden 1978). Additionally, as same as species classification, population study to understand genetic diversity in *Goodyera* is quite important for the species conservation and its sustainable management of gene resource, since many of the species are on the decrease in the population number and size even now.

In Japan, more than ten species of *Goodyera* have been recorded (Sera 1990). Of these, *Goodyera foliosa* (Lindley) Benth. ex C. B. Clarke shows intraspecific morphological variation, and has two varieties in Japan. *Goodyera foliosa* (Lindley) Benth. var. *laevis* Finet is common type of the Japanese species, and is distributed from the north main island (Hokkaido) to the south main island (Kyushu) in the Japanese archipelago, whereas another variety, *Goodyera foliosa* (Lindley) Benth. var. *commelinoides* (Fukuyama) F. Maekawa, is native to only Kyushu island and further down south small islands belonging to Kagoshima, Okinawa Prefectures and the Tokyo metropolitan area, Japan. Moreover, in the previously investigated individuals reported by Tanaka (1965) and Sera (1990), *G. foliosa* var. *laevis* has an intraspecific polyploidy, which is generally considered to play a role to promote the geographic segregation explained by their adaptive selection to environments (Ohi *et al.* 2003). The cytotypes with different ploidy levels of *G. foliosa* var. *laevis* are commonly found in south part of the largest main island (Honshu) in Japan, especially in Hiroshima Prefecture (Sera 1990). However, even though cytological studies have been carried out to see chromosome differentiation among related taxa, there is no report of molecular investigation for estimating genetic diversity in the populations with different cytotypes of this Japanese variety.

---

\*Contribution from the Hiroshima Botanical Garden No.95

1) Hiroshima Botanical Garden

2) Graduate School of Agriculture, Tokai University

3) Department of Plant Science, School of Agriculture, Tokai University

Bulletin of the Hiroshima Botanical Garden, No.30 : 7-14, 2012

Random amplified polymorphic DNA (RAPD) method for DNA amplification (Williams *et al.* 1990) has been widely used as a means of generating genetic markers in many organisms. RAPD technique could identify genotypes directly and help to mitigate complications arising from earlier cytological and morphological studies (Das 2008). Moreover, RAPD method is also used to collect information on the levels and patterns of population genetic diversity in wild plants, because knowledge of genetic diversity is the baseline for conservation (Geburek 1997).

In this study, molecular analysis was carried out using random primers to infer the characteristics of RAPD generated DNA fragments and to investigate genetic diversity of six populations of *G. foliosa* var. *laevis* in Hiroshima Prefecture, Japan.

## Materials and Methods

### Plant materials

The plant materials used are listed in Table 1. To extract total genomic DNA, leaf materials were obtained from 30 plant individuals collected. All of the plants used here were cultivated in the Laboratory of Plant Environment Science, Department of Plant Science, School of Agriculture, Tokai University.

Table 1. Collection data for *Goodyera foliosa* var. *laevis* distributed in Hiroshima Prefecture

Accession number for collection date and site	Individual serial numbers in the accession	Abbreviation of population	Locality	Geographical ordinates	Altitude (m)
TK03533C01	03, 04	HID	Saeki-ku, Hiroshima City	34°23'54"N, 132°20'12"E	150
TK03533C02	I01, I03, II02, II03, A01	TSK	Taisyakukyo, Jinsekikougen-tyo	34°51'31"N, 133°12'51"E	500
TK03533C03	II01, II03, III04, IV02, IV03, V01, A01	KMN	Kumano, Saijo-tyo, Syobara City	35°01'31"N, 133°05'01"E	600
TK03533C04	01, 03, 05, 07, 08	EBS	Mt. Tateeboshiyama, Saijo-tyo, Syobara City	35°02'56"N, 133°04'03"E	790
TK03544C05	I02, I03, I04, II03, II04	GRY	Mt. Garyuzan, Geihoku, Kitahiroshima-tyo	34°41'13"N, 132°11'58"E	1000
TK03557A01	I01, I02, I03, II01, II03, II05	ETJ	Okimi-tyo, Etajima City	34°13'32"N, 132°11'58"E	160

### DNA extraction

Total genomic DNA extraction was followed mainly Hoshi *et al.* (1994). The samples were ground into powder with liquid nitrogen and homogenized in the buffer containing 1 M Tris (pH 8.0), 20 mM EDTA, 1.4 M NaCl, 2% cetyltrimethylammonium bromide and 0.5% mercaptoethanol. The homogenates were extracted three times with an equal volume of chloroform-isoamyl alcohol (24: 1) for 15 min each and the DNAs were precipitated with an equal volume of isopropyl alcohol at room temperature.

### RAPD amplification

RAPD amplification was followed the instructions of RAPD decamer Kit (Operon technologies, Alamenda, CA, USA). To optimize the polymerase chain reaction (PCR) amplification conditions, experiments were carried out with varying concentrations of DNA template, primers, and Taq DNA polymerase. According to previous RAPD work (Tae et al. 1999), two primers of OPA-01 and OPB-05 were selected from the series of Operon technologies (Alamenda, CA, USA).

Amplification was performed on a PCR thermalcycler, Program Temp Control System (Astec, PC-708) with 20  $\mu$ l reaction mixtures containing 10 ng of template DNA, 0.2 mM of each dNTPs (dATP, dTTP, dCTP and dGTP), 0.5 units of Taq DNA polymerase (Toyobo, TAP-211), 10 pmol of primer, 10 mM Tris-HCl, 50 mM KCl and 1.5 mM MgCl<sub>2</sub>. The amplification regime was performed with the following programmes: 94°C for 5 min followed by 45 cycles with 94°C for 30 sec, 42°C for 30 sec, 72°C for 1 min, and a final extension at 72°C for 10 min. Amplified PCR products were performed onto an agarose (1.0% w/v) gel (Nacalai) electrophoresis (iMyrun IMR-201) in 0.5 $\times$ TAE buffer at 75 V for 50 min. The gel was visualized by ethidium bromide staining and photographed under UV illuminater (Funakoshi, NTM-20).

### Data analysis

All amplifications were repeated thrice in order to confirm the reproducible amplification of scored fragments. Second and third reproducible bands were scored for the construction of the data matrix. The marked changes observed in RAPD profiles (disappearance and/or appearance of bands in comparison with untreated control treatments) were evaluated. Each gel of RAPD was analyzed by scoring present (1) or absent (0) bands. The pooled data matrices were entered into the StatPartner version 2.0 package. A dendrogram was constructed by employing UPGMA (Unweighted Pair Group Method with Arithmetic Average) (Sokal and Sneath 1963) to group individual into discrete clusters.

## Results

The potential of RAPD fingerprinting for genetic differentiation within *Goodyera foliosa* var. *laevis* was well demonstrated with amplification of purified genomic DNA of 30 individuals of collected from six populations in Hiroshima Prefecture (Fig. 1).

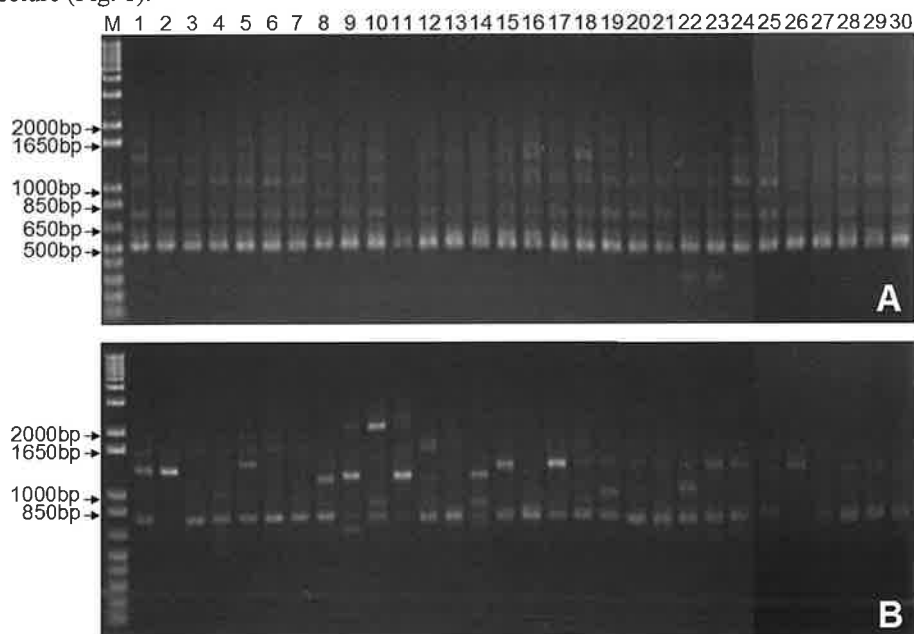


Fig. 1. Random amplified polymorphic DNA of *Goodyera foliosa* var. *laevis* in Hiroshima prefecture, Japan. A, Primer OPA-01. B, Primer OPB-05. Lane M, Marker 1 kb DNA Ladder. 1-2, HID. 3-7, TSK. 8-14, KMN. 15-19, EBS. 20-24, GRY. 25-30, ETJ. Population abbreviations are defined in Table 1 and strain order is the same as that of Table 2.

Using two decamer random primers of OPA-01 and OPB-05, more than six RAPD bands were not obtained in this study. In all, 163 reproducible DNA fragments were amplified, and each primer amplified varying numbers of the fragments. OPA-01 primer generated 98 reproducible bands in the range of 500-1650 bp, while OPB-05 primer generated 65 reproducible bands in the range of 650-2000 bp.

In each population, several bands amplified by OPA-01 primer were shown in the range of 500-1400 bp in KMN and TSK, and 500-1650 bp in EBS, ETJ, GRY and HID. In contrast, one to four bands amplified by OPB-05 primer were shown in the range of 650-1400 bp in KMN, 800-1400 bp in ETJ and HID, 800-1650 bp in GRY, 800-2000 bp in TSK, and 850-1650 bp in EBS.

Table 2. Data matrix of RAPD data for Hiroshima Prefecture, Japan (0, absent; 1, present. Population abbreviations are defined in Table 1)

Primer	bp	Population and individual number																														
		HID				TSK				KMN					EBS					GRY				ETJ								
		03	04	I01	I03	I02	I03	A01	I01	I03	I04	IV02	IV03	V01	A01	01	03	05	07	08	I02	I03	I04	I03	I04	I01	I02	I03	I01	I03	I05	
OPA-01	1650	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	1	1	1	1	0	0
	1400	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	1	1	0	1	1	1	
	1000	0	0	1	1	0	0	1	0	0	0	0	0	1	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	
	700	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	1	1	0	0	
	550	1	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	500	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	
OPB-05	2000	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1650	0	0	0	0	1	1	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	0	0	0	0	0	0	0	
	1550	0	0	1	0	0	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	
	1400	1	1	0	0	1	0	0	1	1	0	1	1	0	1	0	1	1	0	0	1	0	1	1	1	1	1	1	0	1	1	
	1100	0	0	0	0	0	0	0	1	1	0	0	0	0	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	
	1000	0	0	0	0	0	0	0	0	0	1	0	0	0	1	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	
	850	0	0	0	0	0	0	0	0	0	0	0	0	0	1	1	1	1	1	0	0	0	0	0	0	0	0	0	0	0	0	
	800	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0	0	0	0	0	1	1	1	1	1	1	1	1	1	1	1	
	650	0	0	0	0	0	0	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	

The major amplicon numbers of OPA-01 and OPB-05 were six (DNA fragments with 500, 550, 700, 1000, 1400 and 1650 bp) and nine (DNA fragments with 650, 800, 850, 1000, 1100, 1400, 1550, 1650 and 2000 bp), respectively (Table 2).

In OPA-01 primer, all individuals of *G. foliosa* var. *laevis* studied here had a distinct band at 500 bp. OPA-01 primer also made clear bands of 700 bp and 1400 bp in most individuals. But DNA bands appeared at 550 bp, 1000 bp and 1650 bp were only observed in a few individuals. In contrast to OPA-01, primer of OPB-05 exhibited a characteristic 850 bp band in EBS population. Five amplicons were shown in KMN and TSK, while only two amplicons were shown in ETJ and HID. RAPD bands generated by OPA-01 primer were more commonly found in all individuals and populations than those of OPB-05.

Pairwise genetic distances among whole individuals of six populations, as revealed by RAPD analysis, are shown in Table 3. The highest value of 2.828 with 7 character differences was found between TSK-I01 and EBS-05. The genetic dissimilarity matrix had many high values of 2.646 between EBS and the other four populations. In particular, the majority of these second large values in the other populations were mainly found in ETJ and GRY populations. Zero-values, which indicated exactly same banding pattern in RAPD analysis, were seen not only within the populations, but also among some populations.

Cluster analysis of the genetic distance values was performed to generate a dendrogram showing overall genetic relatedness among *G. foliosa* var. *laevis* individuals (Fig. 2). Using 30 individuals obtained from the six populations, four main clades were found in the cluster tree. One of these clades consisted of all five individuals collected from EBS population, but others were not.



## Discussion

A relationship between genetic difference and plant population in this genus has been noticed by a few researchers (Tanaka 1965, Kallunni 1976, Wong and Sun 1999). Kallunni (1976) investigated population diversity of *G. tessellata* in North America, and suggested that high morphological variation in this species was due to hybrid origin with polyploidization event. Wong and Sun (1999) reported that Hong-Kong's populations of *G. procera* showed a wide genetic range without any correlation between genetic diversity and geographic distance. In our study, population clustering based on Nei's genetic identities (Nei 1972) generated from RAPD data was similar to the previous results of Wong and Sun (1999). Except for EBS population (Mt. Tateeboshiyama, Shyobara City), a significant relationship was not found between genetic difference and population (Fig. 2). The clade consisting only of EBS individuals was located at basal position in RAPD tree, and each of the individuals had a long genetic distance. Thus, it can be speculated that population of Mt. Tateeboshiyama kept high and unique genetic diversity.

Our RAPD result showed that most individuals in each population were placed in different main clades with long terminal branches, strongly indicating all populations had high genetic diversity. As researched areas in our study, previous cytogenetic work has interesting information about interspecific polyploidy (Sera 1990). Two cytotypes with different chromosome numbers with  $2n=28$  and  $2n=56$  were found in *Goodyera foliosa* var. *laevis* in Hiroshima Prefecture. Moreover, the populations distributed more west gave chromosome number of  $2n=56$ . Thus, these results indicated that polyploidal chromosome number of *G. foliosa* var. *laevis* derived from more east population of same varieties with a lower chromosome number and high genetic diversity.

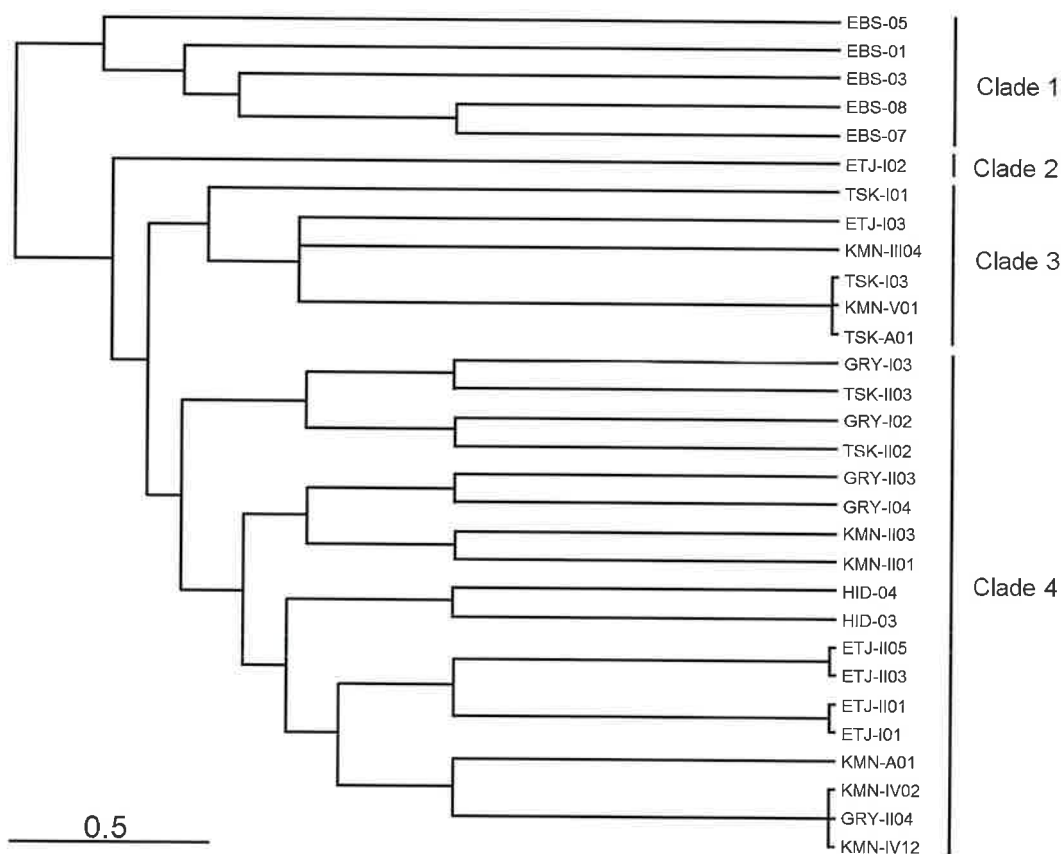


Fig. 2. UPGMA cluster tree of 30 individuals of *Goodyera foliosa* var. *laevis* from six populations in Hiroshima Prefecture, Japan. The scale bar indicates a genetic distance. Population and individual abbreviations are defined in Table 1.

Getting a better understanding of genetic diversity is very important for formulating comprehensive conservation plans (Hamrick 1983, Falk and Holsinger 1991, Loescheke et al. 1994, Geburek 1997). In spite of the threat of extinction, there is little information about genetic diversity of native orchid plant species. The levels and patterns of genetic variation using allozyme and RAPD analyses were provided for wild orchid conservation strategy in Hong-Kong's populations (Wong and Sun 1999). In the comparison with allozyme diversity, higher levels of genetic variation were detected at the RAPD. Therefore, our comparative population study using RAPD method can help us figure out baseline for conservation of the genus *Goodyera* in Hiroshima Prefecture.

Further examinations with other PCR-based molecular markers in many more populations were necessary to clarify the population relationship in this species.

### Acknowledgments

We wish to express our gratitude to Mr. K. Hitotsubashi, a member of volunteer group of the Hiroshima Botanical Garden, for co-operating to collect samples.

### References

- Brieger, F. G. 1974-1975. Unterfamilie Neottioideae, *In Die Orchideen* 3rd. ed., pp. 284-358. Paul Parey, Berlin.
- Das, A. B. 2008. Assessment of genetic diversity and phylogenetic analysis of 'Star Cactus' (*Astrophytum*) through chromosome and RAPD markers. *Cytologia* 37: 179-188.
- Dressler, R. L. 1981. *The Orchid. Natural history and classification*. 344pp. Harvard Univ. Press, Cambridge, Mass.
- Dressler, R. L. and Dodson, C. H. 1960. Classification and phylogeny in the Orchidaceae. *Ann. Missouri Bot. Gard.* 47: 25-68.
- Falk, D. A. and Holsinger, K. 1991. Genetic diversity of rare plants. Oxford University Press, New York, NY.
- Geburek, T. 1997. Isozymes and DNA markers in gene conservation of forest trees. *Biodivers. Conserv.* 6: 1639-1654.
- Hamrick, J. L. 1983. The distribution of genetic variation within and among natural plant populations. *In* Schonewald-Cox, C. M., Chambers, S. M., MacBryde, B. and Thomas, W. L. (eds.). *Genetics and conservation*, pp.335-348. Benjamin/Cummings, Menlo Park, CA.
- Holttum, R. E. 1964. *Flora of Malaya*. vol. 1: *Orchids of Malaya*. 3rd. ed. 771pp. Government Printing Office, Singapore.
- Hoshi, Y., Hizume, M. and Kondo, K. 1994. Genomic *in situ* hybridization to improve a hypothesis on natural-hybrid origin of the hexaploid *Drosera spathulata* 'Kansia type'. *La Kromosomo* II-75-76: 2619-2623.
- Kallunki, J. A. 1976. Population studies in *Goodyera* (Orchidaceae) with emphasis on the hybrid origin of *G. tessellata*. *Brittonia* 28: 53-75.
- Loescheke, V., Tomiuk, J. and Jain, S. K. 1994. Introductory remarks: genetic and conservation biology. *In* V. Loescheke et al. (eds.). *Conservation genetics*, pp.3-8. Birkhauser Verlag, Basel.
- Maekawa, F. 1971. *The wild orchids of Japan in Color*. 495pp. Seibundo Shinkosha, Tokyo. (In Japanese).
- Maekawa, F. 1978. Chapt. 5. *Vexillabium yakushimense*. *In* The endemic plants of Japan, pp.75-91. Tamagawa Univ. Press, Tokyo. (In Japanese).
- Nei, M. 1972. Genetic distance between populations. *Amer. Naturalists* 106: 283-292.
- Ohi, T., Kajita, T. and Murata, J. 2003. Distinct geographic structure as evidenced by chloroplast DNA haplotypes and ploidy level in Japanese *Aucuba* (Aucubaceae). *Am. J. Bot.* 90: 1646-1653.
- Satomi, N. 1982. The Orchidaceae. *In* Satake, Y., Ohwi, J., Kitamura, S., Watari, S. and Tominari, T. (eds.). *Wild flowers of Japan herbaceous plants (including Dwarf Subshrubs) I*, pp.187-235. Heibonsha Ltd., Tokyo. (In Japanese).
- Schlechter, R. 1926. Das System der Ordhidaceen. *Notizbl. Bot. Gart. Mus. Berlin-Dahlem* 9: 563-591.



- Seidenfaden, G. 1978. Orchid genera in Thailand. Dansk Bot. Arkiv. 32: 1-195.
- Sera, T. 1990. Karyomorphological studies on *Goodyera* and its allied genera in Orchidaceae. Bull. Hiroshima Bot. Gard. 12: 71-144.
- Sokal, R. R. and Sneath, P. H. A. 1963. Principles of Numeric Taxonomy. 359pp. Freeman, San Francisco.
- Tae, K. H., In, D. S., H. Y. Bae, and Ko, S. C. 1999. Relationship of the Korean *Goodyera* (Orchidaceae) using random amplified polymorphic DNAs analysis. Kor. J. Plant Tax. 29: 169-181.
- Tanaka, R. 1965. Intraspecific polyploidy on *Goodyera maximowicziana* Makino. La Kromosomo 60: 1945-1950.
- Williams, J. G. K., Kublick, A. R. K., Livak, J., Rafalsky, J. and Tingey, S. V. 1990. DNA polymorphism amplified by arbitrary primers are useful as genetic markers. Nucl. Acids Res. 18: 6531-6535.
- Wong, K. C. and Sun, M. 1999. Reproductive biology and conservation genetics of *Goodyera procera* (Orchidaceae). Am. J. Bot. 86: 1406-1413.

## 広島県に自生するアケボノシュスランを用いた RAPD 解析

世羅徹哉<sup>1)</sup>, 西田知世<sup>2)</sup>, 石田源次郎<sup>1)</sup>, 星良和<sup>3)</sup>

### 要 約

広島県に自生するアケボノシュスラン (*Goodyera foliosa* (Lindley) Benth. var. *laevis* Finet) の遺伝的多様性を調査するため、県内 6 箇所から採取した 30 個体の RAPD (Random Amplified Polymorphic DNA) 解析を行った。PCR 増幅に用いた 2 種類のランダムプライマーで個体間多型を示す DNA 断片が増幅された。得られた多型 DNA をもとに遺伝的距離を求めた結果、帝釈峡と立烏帽子山の個体間で見られた値がもっとも高い数値を示した。クラスター解析の結果から、立烏帽子山の個体群は独自かつ高い遺伝的多様性を保っていることが推察された。

- 
- 1) 広島市植物公園
  - 2) 東海大学大学院農学研究科
  - 3) 東海大学農学部応用植物科学科

## Karyomorphological studies of six species of subtribe *Catasetinae*, *Orchidaceae*

Shuichi Hamatani <sup>1)</sup> and Mikio Aoyama <sup>2)</sup>

### Abstract

The karyomorphological observations were carried out on six species in three genera (*Catasetum*, *Cycnoches* and *Mormodes*) of subtribe *Catasetinae*, *Orchidaceae* cultivated in the Hiroshima Botanical Garden.

In the all six species, the nuclei at resting stage were observed as the complex chromocenter type, and the karyotypes at mitotic prophase were observed as the interstitial type.

The chromosome numbers of *Catasetum tenebrosum* ( $2n=54$ ) and *Mormodes sinuata* ( $2n=54$ ) were reported here for the first time, and *Catasetum cernuum* ( $2n=54$ ), *C. integerrinum* ( $2n=54$ ), *C. viridiflavum* ( $2n=54$ ) and *Cycnoches ventricosum* ( $2n=68$ ) were redocumented. It was suggested that the five species with chromosome number of  $2n=54$  had basic chromosome number of  $x=27$  and the one species with chromosome number of  $2n=68$  had basic chromosome number of  $x=34$ .

The karyotypes at mitotic metaphase were symmetrical due to the centromeric position on the all six species studied. The karyotypes of four species of *Catasetum* and *Cycnoches ventricosum* were gradual, though that of *Mormodes sinuata* was bimodal due to the chromosome length.

### Introduction

The subtribe *Catasetinae*, tribe *Cymbidieae*, the *Orchidaceae* consists of 194 species in five genera (*Catasetum*, *Clowesia*, *Cycnoches*, *Dressleria* and *Mormodes*) in which the most species are distributed in tropical America (Dressler 1993).

The chromosome numbers of 30 species in the subtribe *Catasetinae* were indicated as  $2n=54$ , 56, 64, 68, ca.108 and ca.162 (Blumenschein 1960, Jones and Daker 1967, Nakata and Hashimoto 1990, Félix and Guerra 2000).

The authors have already studied about the chromosomes of tribe *Cymbidieae* (Aoyama 1989). In this study, karyomorphological observations of six species of subtribe *Catasetinae* cultivated in the Hiroshima Botanical Garden were held for enhancing the information about chromosomes.

### Materials and Methods

The six species observed in this study were listed in Table 1. They were in three genera in the subtribe *Catasetinae* and cultivated in the Hiroshima Botanical Garden.

The observation of chromosomes was made by the aceto-orcein squash method. The active root tips were immersed in 0.002M 8-hydroxyquinoline at 15°C for four hours. Then, they were fixed in acetic alcohol (1:3) at 5°C for 24 hours. The fixed materials were hydrolyzed in a 1:2 mixture of 45% acetic acid and 1N HCl at 60°C for 30 seconds. Finally, the materials were squashed and stained in 2% aceto-orcein.

The observations on chromosome morphology were made in nuclei at resting stage, and chromosomes at mitotic prophase and metaphase stages. The types of nuclei at resting stage and chromosomes at mitotic prophase were classified according to Tanaka (1971, 1980), and at mitotic metaphase, they were classified according to Levan *et al.*

---

\*Contribution from the Hiroshima Botanical Garden No.96

1) The Hiroshima Botanical Garden

2) Botanical Garden, Technical Center, Hiroshima University

Bulletin of the Hiroshima Botanical Garden, No.30 : 15-30, 2012

(1964).

### Results

The pictures of the nuclei at resting stage, the chromosomes at mitotic prophase and metaphase, the drawing of the chromosomes at mitotic metaphase and the chromosomes arrangement according to their length and pairing of each species were organized into one figure (Fig. 1-6). The chromosome numbers of all species studied were shown in Table 1. The measurements of chromosome length were described in Table 2-7.

Table 1. Chromosome numbers of the six species of *Catasetinae* studied

Species	HBG* accession number	Chromosome numbers		References
		Present count (2n)	Previous count (2n)	
<i>Catasetum</i>				
<i>cernuum</i> (Lindl.) Rchb.f.	2533	54	54	Jones & Daker 1967
			56	Blumenschein 1960
<i>integerrimum</i> Hook.	2539	54	54	Jones & Daker 1967
<i>tenebrosum</i> Kranzl.	1467	54		
<i>viridiflavum</i> Hook.	3149	54	54	Jones & Daker 1967
<i>Cycnoches</i>				
<i>ventricosum</i> Batem.	3148	68	68	Jones & Daker 1967
<i>Mormodes</i>				
<i>sinuata</i> Rchb.f. & Warm.	3618	54		

\*: Hiroshima Botanical Garden

1) *Catasetum cernuum* (Lindl.) Rchb.f., HBG2533, Tables 1 and 2, Fig. 1.

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 1A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 1B).

The chromosome number of  $2n=54$  was counted at mitotic metaphase (Table 1, Fig. 1C-E). This number was correspondent to the previous report by Jones and Daker (1967).

The chromosomes at mitotic metaphase varied in length from 2.23 to 0.85  $\mu\text{m}$  (Table 2). In the chromosome complement, 54 chromosomes showed a gradual decrease in length. Among the complement of the 54 chromosomes, 49 chromosomes had their centromeres at the median regions (m) and five chromosomes (Nos. 6, 10, 11, 23 and 24) had their centromeres at the submedian regions (sm).

Thus, this species showed a gradual and symmetric karyotype.

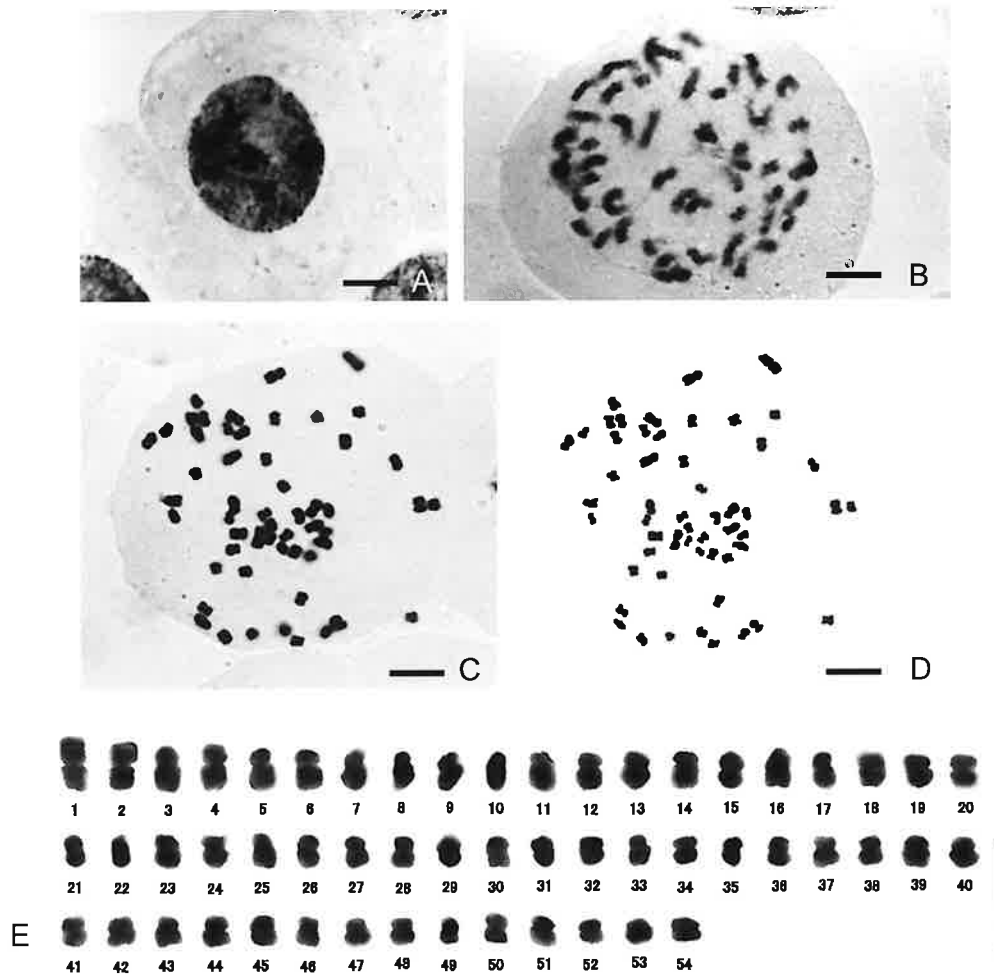


Fig. 1. *Catasetum cernuum* (Lindl.) Rchb.f., HBG2533,  $2n=54$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate  $5\mu\text{m}$ .

**2) *Catasetum integerrimum* Hook., HBG2539, Tables 1 and 3, Fig. 2.**

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 2A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 2B).

The chromosome number of  $2n=54$  was counted at mitotic metaphase (Table 1, Fig. 2C-E). This number was correspondent to the previous report by Jones and Daker (1967).

The chromosomes at mitotic metaphase varied in length from 2.79 to 0.87  $\mu\text{m}$  (Table 3). In the chromosome complement, 54 chromosomes showed a gradual decrease in length. Two chromosomes (Nos. 9 and 10) had satellites at the terminal regions of their long arms. Among the complement of the 54 chromosomes, one chromosome (No. 47) had its centromere at the median point (M), 23 chromosomes (Nos. 1-4, 7-9, 13-16, 25-28, 31, 32, 37-40, 44 and 45) had their centromeres at the median regions, 21 chromosomes (Nos. 5, 6, 10-12, 17-20, 22-24, 29, 30, 33-36 and 41-43) had their centromeres at the submedian regions and one chromosome (No. 21) had its centromere at the subterminal region (st), and in eight chromosomes (Nos. 46 and 48-54), centromeres did not observed in this study.

Thus, this species showed a gradual and symmetric karyotype.

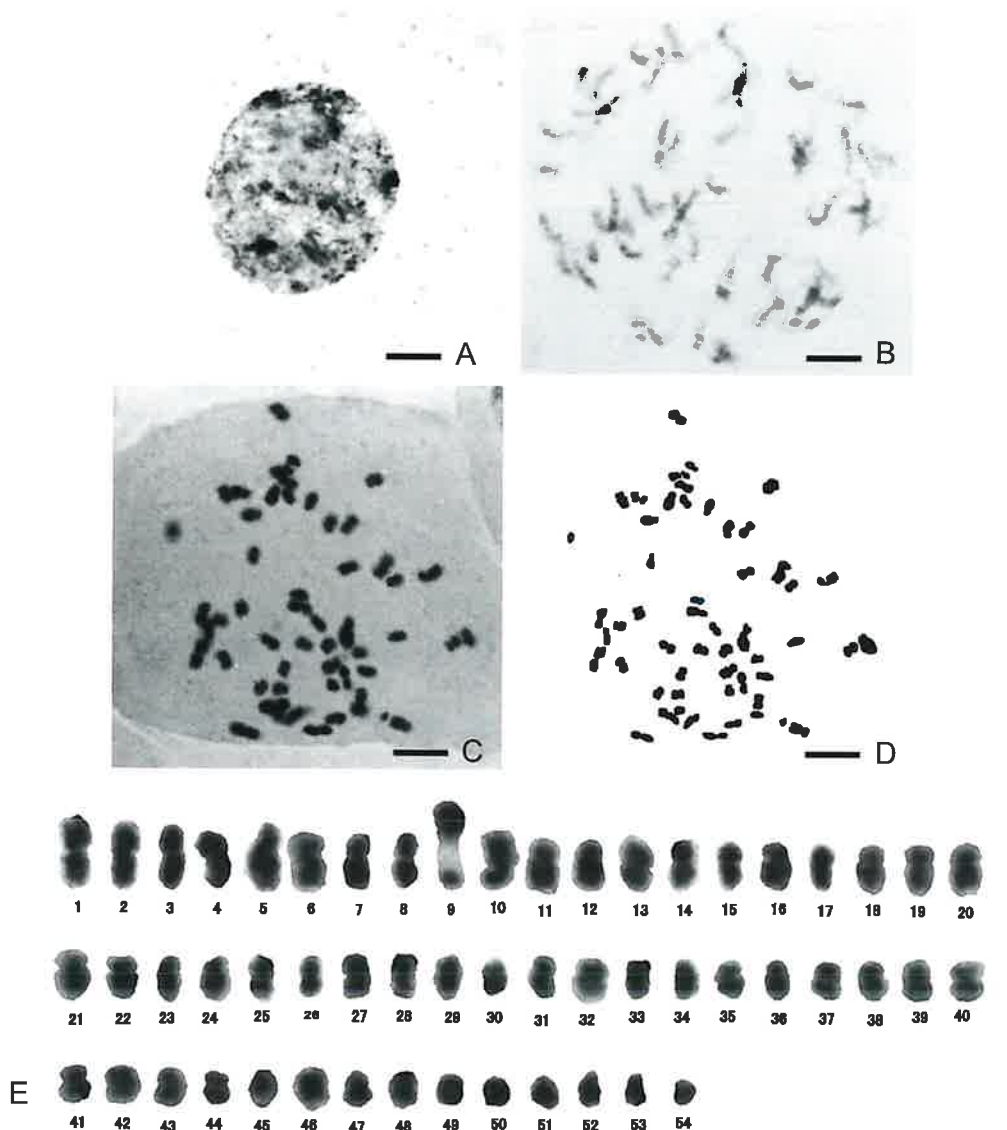


Fig. 2. *Catasetum integerrimum* Hook., HBG2539,  $2n=54$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate 5  $\mu\text{m}$ .

### 3) *Catasetum tenebrosum* Kraenzl., HBG 1467, Tables 1 and 4, Fig. 3.

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 3A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 3B).

The chromosome number of  $2n=54$  was counted at mitotic metaphase (Table 1, Fig. 3C-E). This was reported here for the first time.

The chromosomes at mitotic metaphase varied in length from 3.24 to 1.15  $\mu\text{m}$  (Table 4). In the chromosome complement, 54 chromosomes showed a gradual decrease in length. Among the complement of the 54 chromosomes, 28 chromosomes had their centromeres at the median regions, 23 chromosomes (Nos. 5, 7-10, 13, 19, 20, 23-26, 29, 30, 37, 38, 41, 42, 45-48 and 54) had their centromeres at the submedian regions and three chromosome (Nos. 14-16) had their centromeres at the subterminal regions.

Thus, this species showed a gradual and symmetric karyotype.

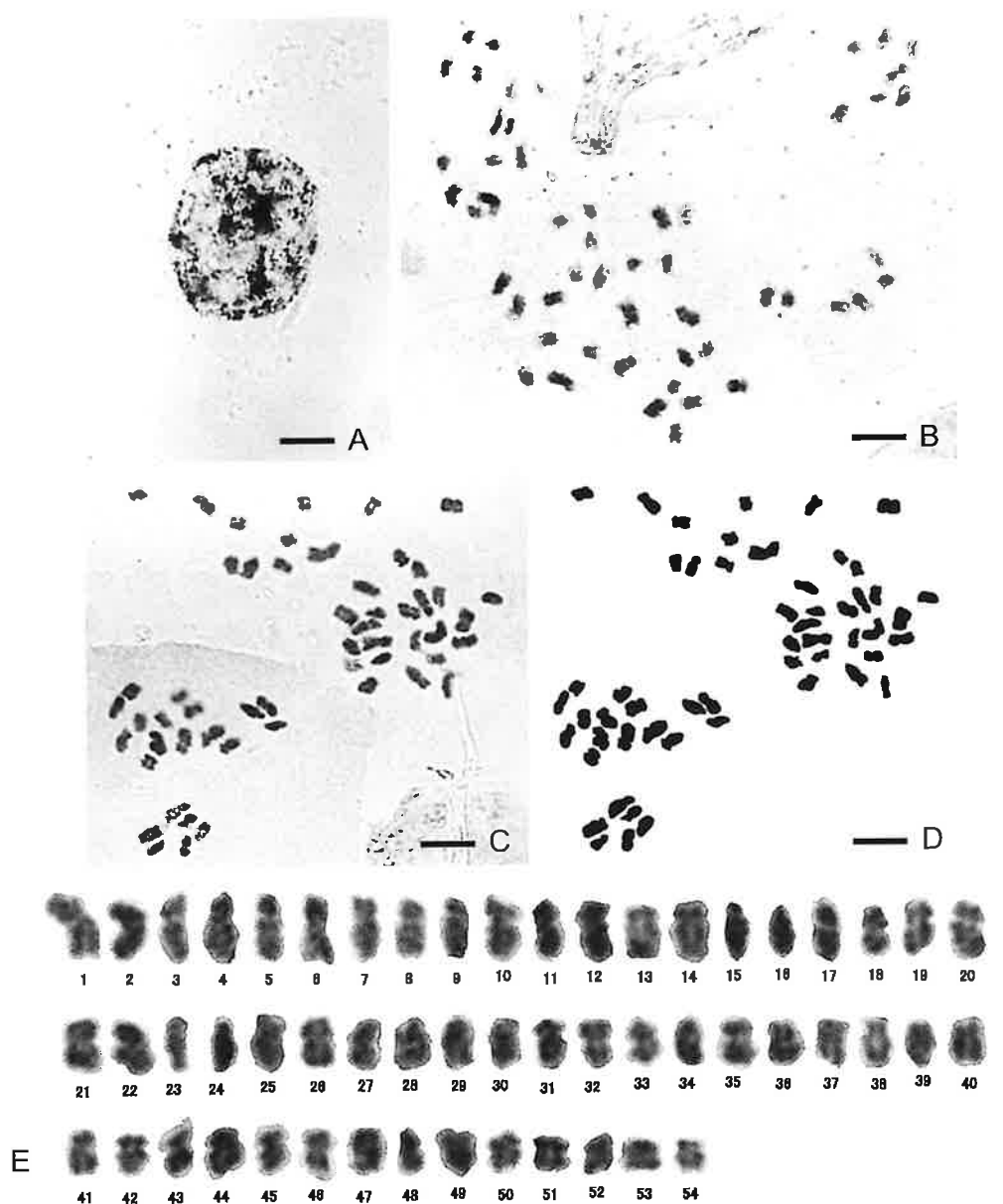


Fig. 3. *Catasetum tenebrosum* Kraenzl., HBG1467,  $2n=54$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate 5  $\mu\text{m}$ .

**4) *Catasetum viridiflavum* Hook., HBG3149, Tables 1 and 5, Fig. 4.**

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 4A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 4B).

The chromosome number of  $2n=54$  was counted at mitotic metaphase (Table 1, Fig. 4C-E). This number was correspondent to the previous report by Jones and Daker (1967).

The chromosomes at mitotic metaphase varied in length from 2.56 to 0.85  $\mu\text{m}$  (Table 5). In the chromosome complement, 54 chromosomes showed a gradual decrease in length. Among the complement of the 54 chromosomes, 31 chromosomes had their centromeres at the median regions and 19 chromosomes (Nos. 5, 6, 10, 19-22, 30, 31, 35-40, 43, 44, 51 and 52) had their centromeres at the submedian regions, and in four chromosomes (Nos. 49, 50, 53 and 54), centromeres did not observed in this study.

Thus, this species showed a gradual and symmetric karyotype.

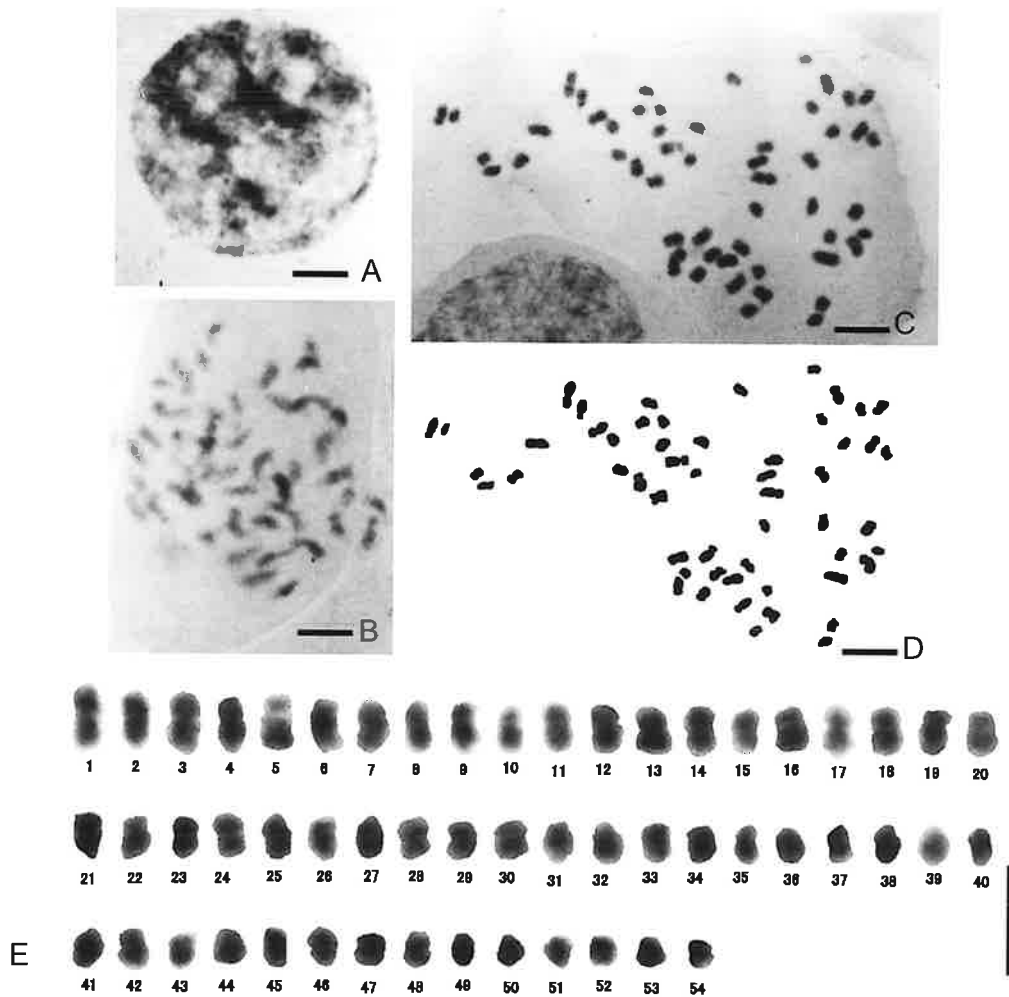


Fig. 4. *Catasetum viridiflavum* Hook., HBG3149,  $2n=54$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate 5  $\mu\text{m}$ .

### 5) *Cycnoches ventricosum* Batem., HBG3148, Tables 1 and 6, Fig.5.

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 5A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 5B).

The chromosome number of  $2n=68$  was counted at mitotic metaphase (Table 1, Fig. 5C-E). This number was correspondent to the previous report by Jones and Daker (1967).

The chromosomes at mitotic metaphase varied in length from 2.65 to 0.74  $\mu\text{m}$  (Table 6). In the chromosome complement, 68 chromosomes showed a gradual decrease in length. Among the complement of the 68 chromosomes, 11 chromosomes (Nos. 3, 4, 37, 38, 41, 42, 47, 48, 54, 57 and 58) had their centromeres at the median regions, 40 chromosomes had their centromeres at the submedian regions and three chromosomes (Nos. 1, 5 and 14) had their centromeres at the subterminal regions, and in 14 chromosomes (Nos. 39, 45, 49, 50, 53, 56, 59, 60 and 63-68), centromeres did not observed in this study.

Thus, this species showed a gradual and symmetric karyotype.

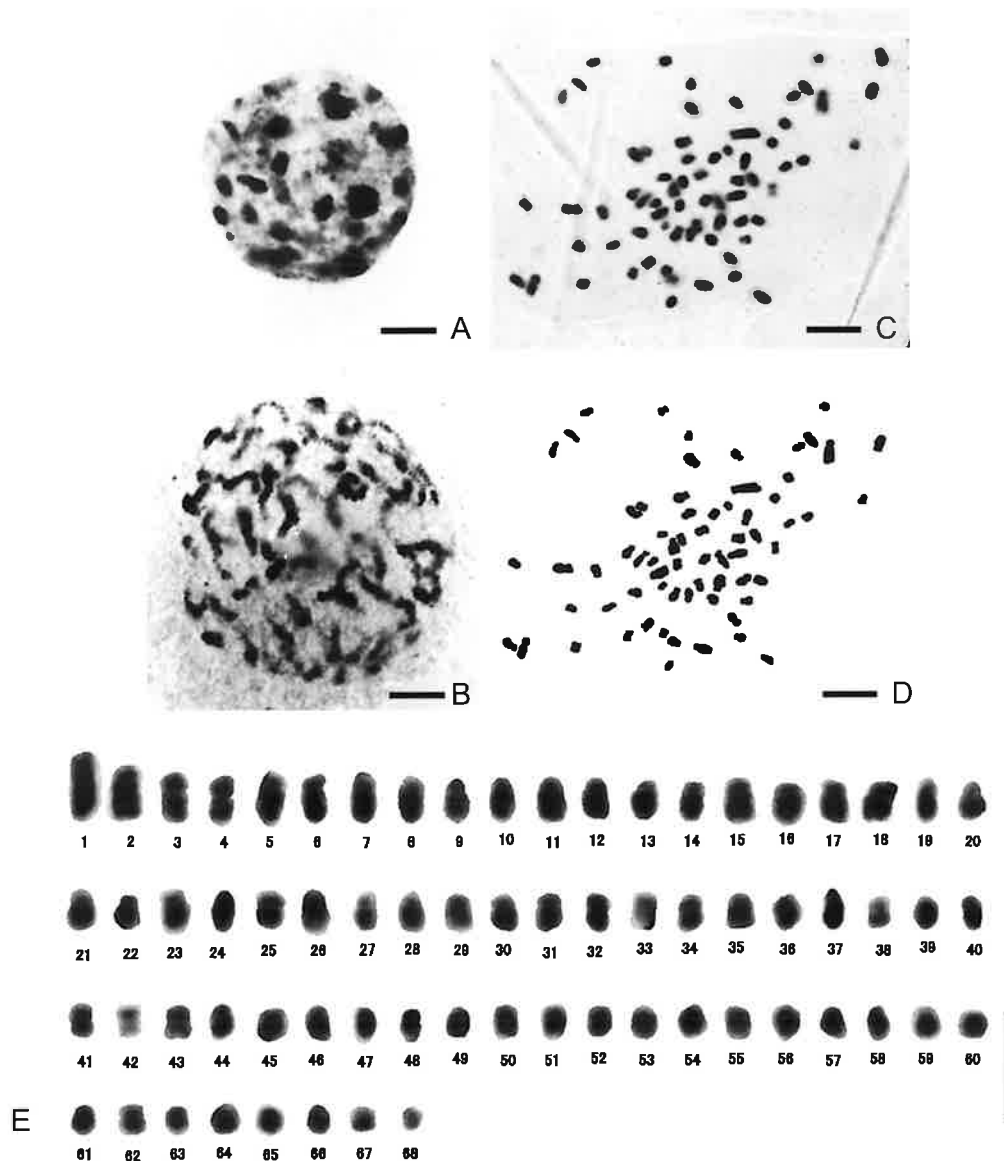


Fig. 5. *Cycnoches ventricosum* Batem, HBG3148,  $2n=68$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate  $5\mu\text{m}$ .



6) *Mormodes sinuata* Rchb.f. & Warm., HBG3618, Tables 1 and 7, Fig. 6.

The nuclei at resting stage were observed as the complex chromocenter type (Fig. 6A).

The karyotype at mitotic prophase were observed as the interstitial type (Fig. 6B).

The chromosome number of  $2n=54$  was counted at mitotic metaphase (Table 1, Fig. 6C-E). This was reported here for the first time.

The chromosomes at mitotic metaphase varied in length from 2.76 to 1.00  $\mu\text{m}$  (Table 7). In the chromosome complement, the two longest chromosomes (Nos. 1 and 2) were distinguished and the other 52 chromosomes showed a gradual decrease in length. Among the complement of the 54 chromosomes, 29 chromosomes had their centromeres at the median regions, 23 chromosomes (Nos. 3, 4, 8, 11, 12, 19-22, 24-26, 31-35, 37-40, 53 and 54) had their centromeres at the submedian regions and the longest two chromosomes (Nos.1 and 2) had their centromeres at the subterminal regions.

Thus, this species showed a bimodal and symmetric karyotype.

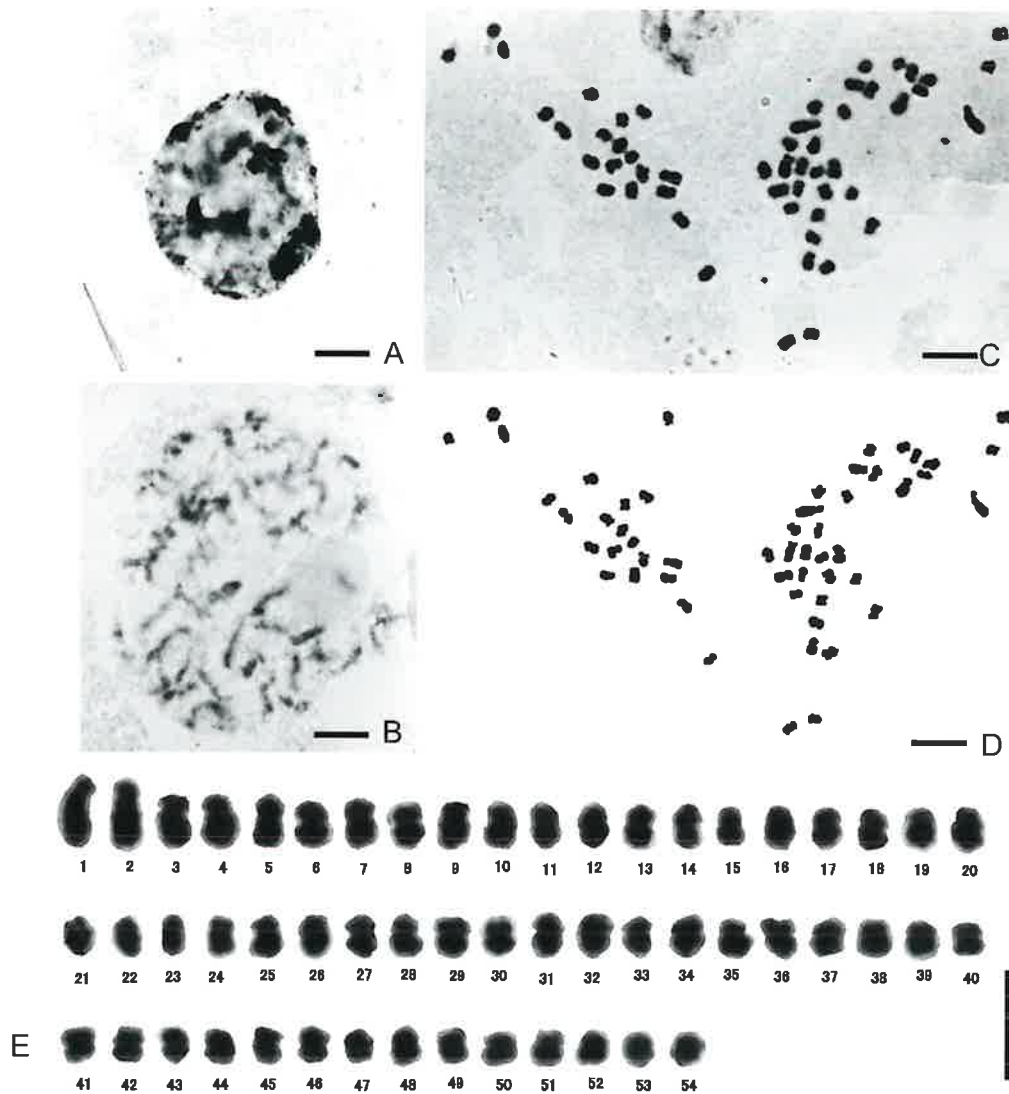


Fig. 6. *Mormodes sinuata* Rchb.f. & Warm., HBG3168,  $2n=54$ .

A: resting stage, B: mitotic prophase, C and E: mitotic metaphase, D: drawing of mitotic metaphase.

Bars indicate  $5\mu\text{m}$ .

## Discussions

In this study, the chromosome numbers of *Catasetum tenebrosum* ( $2n=54$ ) and *Mormodes sinuata* ( $2n=54$ ) were new reports. Those of the other four species supported previous counts.

All four species of genus *Catasetum* showed similar karyotypes of the complex chromocenter type at resting stage and the interstitial type at mitotic prophase. They were consistent each other on their chromosome number of  $2n=54$  and the gradual and symmetric karyotypes at mitotic metaphase. Eight chromosomes of *C. integerrimum* and four chromosomes of *C. viridiflavum* did not show their centromeres, it was speculated that it was caused by the unclear pictures of the small chromosomes.

The chromosome numbers of  $2n=54$ , 56, ca.108 and ca.162 were counted from 18 species of *Catasetum* (Blumenschein 1960, Jones and Daker 1967, Félix and Guerra 2000). Jones and Daker (1967), and Félix and Guerra (2000) mentioned that *Catasetum* showed basic chromosome number of  $x=27$ , and the results of this study supported it.

*Mormodes sinuata* showed similar characters of the chromosomes with *Catasetum* species in this study, i.e. the complex chromocenter type at resting stage, the interstitial type at mitotic prophase, the symmetric karyotype at mitotic metaphase and the chromosome number of  $2n=54$ . The chromosome number of  $2n=54$  were previously counted from three species of *Mormodes* (Jones and Daker 1967, Nakata and Hashimoto 1990). Jones and Daker (1967), and Félix and Guerra (2000) noticed that *Mormodes* showed the basic chromosome number of  $x=27$  and the results of this study supported it. Besides, *Mormodes* showed a bimodal karyotype at mitotic metaphase though *Catasetum* showed a gradual karyotype. It would be one of the points to distinguish between two genera.

*Cycnoches ventricosum* showed different features from other five species of two genera in this study. The chromocenters of *Cycnoches* at resting nuclei were relatively observed more clear and larger than those of *Catasetum* and *Mormodes*. The chromosome number of  $2n=68$  was different from other five species. The average of arm ratios of this species was higher than those of other five species. The chromosome numbers of  $2n=64$  and 68 were previously counted in four species of *Cycnoches* (Jones and Daker 1967). Jones and Daker suggested that the basic chromosome number of *Cycnoches* was  $x=32$  or 34 and there were karyomorphologically distinctions between *Cycnoches* and *Catasetum*. Present report did not show contradictory results for the previous reports.

In the recent molecular studies, it was advocated that subtribe Catasetinae took in more three genera of *Cyrtopodium*, *Galeandra* and *Grobya* (Chase 2012). Many species still remain to be cytological studies in subtribe Catasetinae. It is necessary to research on the species of subtribe Catasetinae including new genera not only by molecular studies but also cytotaxonomical studies.

## Acknowledgement

We thank to Mr. Daizo Araki for reading the manuscripts and giving us advices.

## References

- Aoyama, M. 1989. Karyomorphological studies in *Cymbidium* and its allied genera, Orchidaceae. Bull. Hiroshima Bot. Gard. 11: 1-121.
- Blumenschein, A. 1960. Numero de cromossomas de algumas espécies de orquideas. Publ. Cien. Univ. São Paulo Inst. Genet. 1: 47-48.
- Dressler, R. L. 1993. Phylogeny and Classification of the Orchid Family. Dioscorides Press. Portland OR.
- Félix, L. P. and Guerra, M. 2000. Cytogenetics and cytotaxonomy of some Brazilian species of Cymbidioid orchids. Genetics and Molecular Biology, 23 (4): 957-978.
- Jones, K. and Daker, M. G. 1967. The chromosomes of orchids, III. Catasetinae Schltr. Kew Bull. 22: 421-427.

- Levan, A., Fredga, K. and Sandberg, A. A. 1964. Nomenclature for centromeric position on chromosomes. *Hereditas* 52: 201-220.
- Nakata, M. and Hashimoto, T. 1990. Cytological studies on phanerogams in southern Peru, III. Chromosomes of some orchid species. *Bull. Natn. Sci. Mus., Tokyo, Ser. B*, 16 (4): 157-169.
- Chase, M. W. 2012. DNA data and orchidaceae systematics : A new phylogenetic classification. <http://www.orchids.co.in/dna-data-orchidaceae.shtml>
- Tanaka, R. 1971. Types of resting nuclei of Orchidaceae. *Bot. Mag. Tolyo*, 84: 118-122.
- Tanaka, R. 1980. The karyotype. (in Japanese). *In* Kihara, H. (ed.), *Plant Genetics* 1: 335-358. Shokabo Co., Tokyo.

## カタセタム亜族（ラン科）6種の核形態学的研究

濱谷修一<sup>1)</sup>・青山幹男<sup>2)</sup>

### 要 約

広島市植物公園で栽培しているラン科カタセタム亜族に含まれる3属 (*Catasetum*, *Cycnoches*, *Mormodes*) の6種について核形態学的観察を行った。

調査した6種全てにおいて、静止期核は複雑染色中央粒型、体細胞分裂前期の核型は介在型として観察された。

*Catasetum tenebrosum* と *Mormodes sinuata* の染色体数  $2n=54$  は初の報告だった。 *Catasetum cernuum*, *C. integerrimum*, *C. viridiflavum* の  $2n=54$ , *Cycnoches ventricosum* の  $2n=68$  は過去の報告を裏付けるものであった。染色体数  $2n=54$  を示した5種は染色体基本数  $x=27$ ,  $2n=68$  の1種は染色体基本数  $x=34$  と示唆された。

調査した全6種において、体細胞分裂中期の核型は動原体の位置に基づく表現は対称的とされた。また、染色体長に基づく表現では *Catasetum* の4種と *Cycnoches ventricosum* は漸減的、*Mormodes sinuata* は二相的とすることができた。

---

1) 広島市植物公園

2) 広島大学技術センター附属植物園

Table 2. Measurements of somatic chromosomes at mitotic metaphase in *Catasetum cernuum*,  $2n=54$ 

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.08+1.15=2.23	3.3	1.06	m
2	1.01+1.07=2.08	3.1	1.06	m
3	0.91+1.02=1.93	2.8	1.12	m
4	0.91+1.02=1.93	2.8	1.12	m
5	0.66+1.07=1.67	2.6	1.62	m
6	0.60+1.07=1.67	2.5	1.78	sm
7	0.68+0.98=1.66	2.4	1.44	m
8	0.56+0.95=1.51	2.2	1.65	m
9	0.57+0.93=1.50	2.2	1.63	m
10	0.42+1.05=1.47	2.2	2.50	sm
11	0.47+1.01=1.48	2.2	2.15	sm
12	0.55+0.91=1.46	2.2	1.65	m
13	0.57+0.86=1.43	2.1	1.51	m
14	0.57+0.86=1.43	2.1	1.51	m
15	0.64+0.77=1.41	2.1	1.20	m
16	0.57+0.82=1.39	2.0	1.44	m
17	0.52+0.85=1.37	2.0	1.63	m
18	0.47+0.77=1.24	1.8	1.64	m
19	0.56+0.74=1.30	1.9	1.32	m
20	0.52+0.68=1.20	1.8	1.31	m
21	0.51+0.78=1.29	1.9	1.53	m
22	0.53+0.68=1.21	1.8	1.28	m
23	0.45+0.78=1.23	1.8	1.73	sm
24	0.45+0.77=1.22	1.8	1.71	sm
25	0.48+0.74=1.22	1.8	1.54	m
26	0.43+0.72=1.15	1.7	1.67	m
27	0.49+0.72=1.21	1.8	1.47	m
28	0.46+0.67=1.13	1.7	1.46	m
29	0.54+0.66=1.20	1.8	1.22	m
30	0.51+0.66=1.17	1.7	1.29	m
31	0.45+0.73=1.18	1.7	1.62	m
32	0.45+0.72=1.17	1.7	1.60	m

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
33	0.42+0.68=1.10	1.6	1.62	m
34	0.40+0.64=1.04	1.5	1.60	m
35	0.46+0.64=1.10	1.6	1.39	m
36	0.44+0.58=1.02	1.5	1.32	m
37	0.48+0.60=1.08	1.6	1.25	m
38	0.46+0.59=1.05	1.5	1.28	m
39	0.43+0.64=1.07	1.6	1.49	m
40	0.43+0.59=1.02	1.5	1.37	m
41	0.46+0.58=1.04	1.5	1.26	m
42	0.42+0.52=0.94	1.4	1.24	m
43	0.42+0.62=1.04	1.5	1.48	m
44	0.45+0.58=1.03	1.5	1.29	m
45	0.46+0.57=1.03	1.5	1.24	m
46	0.46+0.54=1.00	1.5	1.17	m
47	0.41+0.59=1.00	1.5	1.44	m
48	0.40+0.57=0.97	1.4	1.43	m
49	0.40+0.60=1.00	1.5	1.50	m
50	0.45+0.48=0.93	1.4	1.07	m
51	0.40+0.57=0.97	1.4	1.43	m
52	0.36+0.53=0.89	1.3	1.47	m
53	0.37+0.49=0.86	1.3	1.32	m
54	0.36+0.49=0.85	1.3	1.36	m

m: The centromere observed at the median region.  
sm: The centromere observed at the submedian region.

Table 3. Measurements of somatic chromosomes at mitotic metaphase in *Catasetum integrinum*,  $2n=54$

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.25 + 1.54=2.79	3.3	1.23	m
2	1.16+1.33=2.49	3.0	1.15	m
3	1.03+1.41=2.44	2.9	1.37	m
4	0.90+1.31=2.21	2.6	1.46	m
5	0.70+1.46=2.16	2.6	2.07	sm
6	0.67+1.42=2.09	2.5	2.12	sm
7	0.83+1.31=2.14	2.6	1.58	m
8	0.82+1.23=2.05	2.5	1.50	m
9	*0.57+0.80+0.46=1.83	2.2	1.29	m
10	*0.56+0.47+0.77=1.80	2.2	2.84	sm
11	0.63+1.18=1.81	2.2	1.87	sm
12	0.65+1.12=1.77	2.1	1.72	sm
13	0.79+1.00=1.79	2.1	1.27	m
14	0.74+1.03=1.77	2.1	1.39	m
15	0.67+1.08=1.75	2.1	1.61	m
16	0.57+0.91=1.48	1.8	1.60	m
17	0.59+1.16=1.75	2.1	1.97	sm
18	0.55+1.07=1.62	1.9	1.95	sm
19	0.53+1.11=1.64	2.0	2.09	sm
20	0.54+1.05=1.59	1.9	1.94	sm
21	0.40+1.22=1.62	1.9	3.05	st
22	0.46+1.02=1.48	1.8	2.22	sm
23	0.45+1.16=1.61	1.9	2.58	sm
24	0.50+1.06=1.56	1.9	2.12	sm
25	0.71+0.90=1.61	1.9	1.27	m
26	0.71+0.88=1.59	1.9	1.24	m
27	0.70+0.85=1.55	1.9	1.21	m
28	0.61+0.91=1.52	1.8	1.49	m
29	0.51+1.03=1.54	1.8	2.02	sm
30	0.44+0.91=1.35	1.6	2.07	sm
31	0.59+0.87=1.46	1.7	1.47	m
32	0.51+0.86=1.37	1.6	1.69	m

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
33	0.46+0.99=1.45	1.7	2.15	sm
34	0.43+0.98=1.41	1.7	2.28	sm
35	0.46+0.95=1.41	1.7	2.07	sm
36	0.50+0.87=1.37	1.6	1.74	sm
37	0.55+0.84=1.39	1.7	1.53	m
38	0.50+0.77=1.27	1.5	1.54	m
39	0.58+0.79=1.37	1.6	1.36	m
40	0.56+0.77=1.33	1.6	1.38	m
41	0.47+0.85=1.32	1.6	1.81	sm
42	0.39+0.76=1.15	1.4	1.95	sm
43	0.46+0.80=1.26	1.5	1.74	sm
44	0.46+0.76=1.22	1.5	1.65	m
45	0.47+0.77=1.24	1.5	1.64	m
**46	1.20	1.4		
47	0.56+0.58=1.14	1.4	1.04	M
**48	1.10	1.3		
**49	1.05	1.3		
**50	0.96	1.1		
**51	0.96	1.1		
**52	0.96	1.1		
**53	0.96	1.1		
**54	0.87	1.0		

\*: Chromosome with secondary constriction  
 \*\*: The centromere was not observed.  
 M: The centromere observed at the median point.  
 st: The centromere observed at the subterminal region.  
 See Table 2 for explanation of the other symbols.

Table 4. Measurements of somatic chromosomes at mitotic metaphase in *Catasetum tenebrosus*,  $2n=54$ 

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.54+1.70=3.24	2.9	1.10	m
2	1.22+1.82=3.04	2.8	1.05	m
3	1.22+1.82=3.04	2.8	1.49	m
4	1.13+1.57=2.70	2.4	1.39	m
5	0.88+1.91=2.79	2.5	2.17	sm
6	1.11+1.60=2.71	2.5	1.44	m
7	0.92+1.76=2.68	2.4	1.91	sm
8	0.75+1.54=2.29	2.1	2.05	sm
9	0.69+1.88=2.57	2.3	2.72	sm
10	0.68+1.72=2.40	2.2	2.53	sm
11	1.00+1.48=2.48	2.2	1.48	m
12	0.92+1.54=2.46	2.2	1.67	m
13	0.63+1.78=2.41	2.2	2.83	sm
14	0.58+1.77=2.35	2.1	3.05	st
15	0.57+1.81=2.38	2.2	3.18	st
16	0.52+1.59=2.11	1.9	3.06	st
17	1.00+1.36=2.36	2.1	1.36	m
18	0.95+1.08=2.03	1.8	1.14	m
19	0.78+1.52=2.30	2.1	1.95	sm
20	0.77+1.45=2.22	2.0	1.88	sm
21	0.93+1.36=2.29	2.1	1.46	m
22	0.94+1.15=2.09	1.9	1.22	m
23	0.60+1.60=2.20	2.0	2.67	sm
24	0.74+1.42=2.16	2.0	1.92	sm
25	0.71+1.42=2.13	1.9	2.00	sm
26	0.76+1.34=2.10	1.9	1.76	sm
27	0.90+1.16=2.06	1.9	1.29	m
28	0.84+1.14=1.98	1.8	1.36	m
29	0.75+1.28=2.03	1.8	1.71	sm
30	0.70+1.24=1.94	1.8	1.77	sm
31	0.76+1.23=1.99	1.8	1.62	m
32	0.70+1.12=1.82	1.6	1.60	m

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
33	0.78+1.06=1.84	1.7	1.36	m
34	0.83+1.01=1.84	1.7	1.22	m
35	0.68+1.14=1.82	1.6	1.68	m
36	0.59+1.00=1.59	1.4	1.69	m
37	0.53+1.28=1.81	1.6	2.42	sm
38	0.50+1.22=1.72	1.6	2.44	sm
39	0.68+1.10=1.78	1.6	1.62	m
40	0.61+1.03=1.64	1.5	1.69	m
41	0.63+1.14=1.77	1.6	1.81	sm
42	0.54+1.02=1.56	1.4	1.89	sm
43	0.70+1.05=1.75	1.6	1.50	m
44	0.66+1.01=1.67	1.5	1.53	m
45	0.57+1.05=1.62	1.5	1.84	sm
46	0.54+1.05=1.59	1.4	1.94	sm
47	0.52+1.06=1.58	1.4	2.04	sm
48	0.54+0.99=1.53	1.4	1.83	sm
49	0.55+0.93=1.48	1.3	1.69	m
50	0.58+0.88=1.46	1.3	1.52	m
51	0.52+0.85=1.37	1.2	1.63	m
52	0.50+0.84=1.34	1.2	1.68	m
53	0.54+0.64=1.18	1.1	1.19	m
54	0.38+0.77=1.15	1.0	2.03	sm

See Table 3 for explanation of symbols.

Table 5. Measurements of somatic chromosomes at mitotic metaphase in *Catasetum viridiflavum*,  $2n=54$

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.15+1.41=2.56	3.2	1.23	m
2	0.96+1.48=2.44	3.0	1.54	m
3	1.06+1.34=2.40	3.0	1.26	m
4	0.98+1.26=2.24	2.8	1.29	m
5	0.74+1.40=2.14	2.6	1.89	sm
6	0.52+1.37=1.89	2.3	2.63	sm
7	0.93+1.09=2.02	2.5	1.17	m
8	0.82+1.10=1.92	2.4	1.34	m
9	0.81+1.10=1.91	2.4	1.36	m
10	0.65+1.18=1.83	2.3	1.82	sm
11	0.70+1.10=1.80	2.2	1.57	m
12	0.71+1.06=1.77	2.2	1.49	m
13	0.81+0.94=1.75	2.2	1.16	m
14	0.81+0.94=1.75	2.2	1.16	m
15	0.73+0.97=1.70	2.1	1.32	m
16	0.68+0.90=1.58	2.0	1.32	m
17	0.75+0.97=1.72	2.1	1.29	m
18	0.78+0.87=1.65	2.0	1.12	m
19	0.58+1.09=1.67	2.1	1.88	sm
20	0.58+1.06=1.64	2.0	1.83	sm
21	0.49+1.13=1.63	2.0	2.30	sm
22	0.41+0.97=1.38	1.7	2.34	sm
23	0.73+0.82=1.55	1.9	1.11	m
24	0.71+0.81=1.52	1.9	1.14	m
25	0.54+0.90=1.44	1.8	1.66	m
26	0.57+0.84=1.41	1.7	1.46	m
27	0.65+0.78=1.43	1.8	1.21	m
28	0.61+0.69=1.30	1.6	1.14	m
29	0.60+0.81=1.41	1.7	1.35	m
30	0.59+0.81=1.39	1.7	1.37	m
31	0.48+0.83=1.31	1.6	1.74	sm
32	0.45+0.83=1.28	1.6	1.83	sm

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
33	0.53+0.76=1.29	1.6	1.42	m
34	0.53+0.74=1.27	1.6	1.40	m
35	0.39+0.89=1.28	1.6	2.26	sm
36	0.43+0.82=1.25	1.5	1.91	sm
37	0.44+0.81=1.25	1.5	1.84	sm
38	0.40+0.81=1.21	1.5	2.00	sm
39	0.40+0.84=1.25	1.5	2.09	sm
40	0.41+0.84=1.25	1.5	2.07	sm
41	0.53+0.72=1.25	1.5	1.37	m
42	0.53+0.73=1.25	1.5	1.37	m
43	0.36+0.87=1.23	1.5	2.42	sm
44	0.37+0.63=1.00	1.2	1.70	sm
45	0.58+0.63=1.21	1.5	1.08	m
46	0.53+0.62=1.15	1.4	1.16	m
47	0.48+0.71=1.19	1.5	1.49	m
48	0.49+0.68=1.17	1.4	1.39	m
**49	1.08	1.3		
**50	0.99	1.2		
51	0.29+0.70=0.99	1.2	2.42	sm
52	0.32+0.65=0.97	1.2	1.99	sm
**53	0.96	1.2		
**54	0.85	1.1		

See Table 3 for explanation of symbols.

Table 6. Measurements of somatic chromosomes at mitotic metaphase in *Cynoches ventricosum*, 2n=68

Chromosome	Length (µm)	Relative length	Arm rati	Form
1	0.63+2.02=2.65	3.0	3.23	st
2	0.55+1.55=2.10	2.4	2.80	sm
3	0.80+1.20=2.00	2.3	1.49	m
4	0.82+1.01=1.83	2.1	1.23	m
5	0.46+1.41=1.87	2.1	3.09	st
6	0.54+1.30=1.84	2.1	2.40	sm
7	0.56+1.27=1.83	2.1	2.26	sm
8	0.57+1.16=1.73	2.0	2.03	sm
9	0.59+1.13=1.72	2.0	1.92	sm
10	0.44+1.15=1.59	1.8	2.60	sm
11	0.44+1.17=1.61	1.8	2.66	sm
12	0.46+1.12=1.58	1.8	2.42	sm
13	0.41+1.15=1.56	1.8	2.82	sm
14	0.36+1.08=1.44	1.6	3.01	st
15	0.46+1.07=1.54	1.7	2.31	sm
16	0.41+1.05=1.46	1.7	2.56	sm
17	0.55+0.97=1.52	1.7	1.78	sm
18	0.44+1.00=1.60	1.6	2.26	sm
19	0.50+0.92=1.43	1.6	1.83	sm
20	0.52+0.89=1.41	1.6	1.73	sm
21	0.43+0.98=1.41	1.6	2.25	sm
22	0.39+0.99=1.38	1.6	2.54	sm
23	0.39+0.97=1.36	1.5	2.51	sm
24	0.40+0.93=1.34	1.5	2.31	sm
25	0.49+0.86=1.35	1.5	1.74	sm
26	0.49+0.85=1.34	1.5	1.75	sm
27	0.40+0.89=1.30	1.5	2.21	sm
28	0.35+0.87=1.22	1.4	2.48	sm
29	0.35+0.94=1.29	1.5	2.73	sm
30	0.36+0.92=1.28	1.5	2.58	sm
31	0.41+0.86=1.27	1.4	2.10	sm
32	0.42+0.85=1.27	1.4	2.01	sm
33	0.45+0.78=1.23	1.4	1.75	sm
34	0.44+0.77=1.21	1.4	1.75	sm
35	0.38+0.85=1.23	1.4	2.25	sm

Chromosome	Length (µm)	Relative length	Arm rati	Form
36	0.30+0.84=1.15	1.3	2.78	sm
37	0.54+0.65=1.19	1.4	1.21	m
38	0.49+0.62=1.11	1.3	1.28	m
**39	1.18	1.3		
40	0.40+0.76=1.16	1.3	1.91	sm
41	0.48+0.69=1.17	1.3	1.45	m
42	0.45+0.71=1.16	1.3	1.59	m
43	0.39+0.77=1.16	1.3	1.95	sm
44	0.39+0.76=1.15	1.3	1.93	sm
**45	1.16	1.3		
46	0.36+0.79=1.15	1.3	2.20	sm
47	0.42+0.69=1.11	1.3	1.66	m
48	0.42+0.65=1.06	1.2	1.55	m
**49	1.10	1.3		
**50	1.06	1.2		
51	0.36+0.70=1.06	1.2	1.97	sm
52	0.36+0.70=1.06	1.2	1.97	sm
**53	1.04	1.2		
54	0.39+0.65=1.04	1.2	1.65	m
55	0.27+0.76=1.03	1.2	2.77	sm
**56	1.05	1.2		
57	0.38+0.60=0.98	1.1	1.59	m
58	0.36+0.60=0.96	1.1	1.65	m
**59	0.98	1.1		
**60	0.94	1.1		
61	0.32+0.65=0.97	1.1	2.01	sm
62	0.34+0.62=0.96	1.1	1.84	sm
**63	0.94	1.1		
**64	0.93	1.1		
**65	0.92	1.0		
**66	0.82	0.9		
**67	0.80	0.9		
**68	0.74	0.8		

See Table 3 for explanation of symbols.



Table 7. Measurements of somatic chromosomes at mitotic metaphase in *Mormodes sinuata*,  $2n=54$

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form	Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	0.64+2.12=2.76	3.4	3.31	st	33	0.46+0.97=1.43	1.8	2.09	sm
2	0.59+2.11=2.70	3.3	3.55	st	34	0.51+0.86=1.37	1.7	1.70	sm
3	0.65+1.49=2.14	2.6	2.31	sm	35	0.51+0.90=1.41	1.7	1.76	sm
4	0.58+1.41=1.99	2.5	2.43	sm	36	0.52+0.84=1.36	1.7	1.62	m
5	0.82+1.26=2.09	2.6	1.53	m	37	0.49+0.85=1.34	1.6	1.74	sm
6	0.77+1.13=1.90	2.3	1.47	m	38	0.46+0.80=1.26	1.6	1.75	sm
7	0.72+1.17=1.89	2.3	1.61	m	39	0.37+0.90=1.27	1.6	2.42	sm
8	0.61+1.04=1.65	2.0	1.71	sm	40	0.39+0.74=1.13	1.4	1.89	sm
9	0.72+1.16=1.87	2.3	1.61	m	41	0.56+0.68=1.24	1.5	1.21	m
10	0.63+0.98=1.61	2.0	1.56	m	42	0.54+0.68=1.22	1.5	1.25	m
11	0.57+1.09=1.66	2.0	1.92	sm	43	0.54+0.70=1.24	1.5	1.30	m
12	0.60+1.03=1.63	2.0	1.70	sm	44	0.47+0.73=1.21	1.5	1.56	m
13	0.73+0.88=1.60	2.0	1.21	m	45	0.48+0.75=1.23	1.5	1.58	m
14	0.70+0.89=1.59	2.0	1.26	m	46	0.48+0.74=1.23	1.5	1.54	m
15	0.74+0.84=1.58	1.9	1.14	m	47	0.47+0.72=1.19	1.5	1.55	m
16	0.64+0.89=1.53	1.9	1.39	m	48	0.45+0.73=1.18	1.5	1.64	m
17	0.60+0.97=1.57	1.9	1.60	m	49	0.52+0.64=1.16	1.4	1.24	m
18	0.58+0.93=1.51	1.9	1.59	m	50	0.54+0.61=1.15	1.4	1.13	m
19	0.48+1.09=1.57	1.9	2.26	sm	51	0.45+0.71=1.15	1.4	1.58	m
20	0.48+1.01=1.49	1.8	2.11	sm	52	0.43+0.70=1.13	1.4	1.64	m
21	0.55+1.01=1.56	1.9	1.82	sm	53	0.33+0.73=1.05	1.3	2.21	sm
22	0.55+0.96=1.51	1.9	1.73	sm	54	0.28+0.72=1.00	1.2	2.57	sm
23	0.59+0.95=1.54	1.9	1.62	m					
24	0.56+0.95=1.52	1.9	1.70	sm					
25	0.56+0.96=1.52	1.9	1.70	sm					
26	0.53+0.95=1.48	1.8	1.78	sm					
27	0.62+0.90=1.52	1.9	1.45	m					
28	0.57+0.83=1.40	1.7	1.44	m					
29	0.57+0.92=1.49	1.8	1.61	m					
30	0.58+0.89=1.47	1.8	1.55	m					
31	0.54+0.94=1.48	1.8	1.74	sm					
32	0.53+0.93=1.46	1.8	1.76	sm					

See Table 2 for explanation of symbols.

## Karyomorphological observation on 14 species of subtribe Stanhopeinae, Orchidaceae\*

Tetsuya Sera<sup>1)</sup>, Mikio Aoyama<sup>2)</sup> and Genjiro Ishida<sup>1)</sup>

### Abstract

Fourteen species of the subtribe Stanhopeinae, Orchidaceae, were karyomorphologically observed by aceto-orcein squash method. Chromosome numbers of 11 species in nine genera were revealed for the first time;  $2n=38$  for *Coeliopsis hyacinthosma* and *Gongora armeniaca*, and  $2n=40$  for *Acineta barkeri*, *Cirrhaea loddigesii*, *Kegeliella atropilosa*, *Paphinia grandiflora*, *Polycycnis barbata*, *Shlimia alpina*, *Stanhopea cirrhata*, *S. guttulata* and *S. pulla*. Karyotypes of all the 14 species were similar to each other; while chromosome features at resting stage and mitotic metaphase showed slight variations. From the above results, it was suggested that three genera of *Cirrhaea*, *Polycycnis* and *Schlimia* were not closely related among the members of subtribe Stanhopeinae.

### Introduction

Karyotype morphology of the living collection at Hiroshima Botanical Garden has been studied (Karasawa 1979, Hashimoto 1982, Sera & Karasawa 1984, Ishida 1990, 2001 Aoyama 1989, Hamatani 2011, etc.). These studies were carried out on Orchidaceae, Araceae, Gesneriaceae, Liliaceae and so on in order to contribute to plant science by revealing the relationship among species and accumulating the basic knowledge about plants.

In this study chromosomal observation has been made on 14 species of 10 genera of Stanhopeinae Benth, one of a subtribe belonging to the tribe Maxillarieae of the Orchidaceae (Dressler 1993). Chromosomes of three species among the 14 examined here have been reported previously, so that the present survey increases our knowledge about plants as well as a series of studies held at the Hiroshima Botanical Garden.

### Material and methods

All the plants examined in this study were grown in cultivation at the Hiroshima Botanical Garden and listed in Table 1.

Chromosome counts and observation were made from the aceto-orcein squash method as same as those of Hamatani and Aoyama (2012). Karyotype morphology in the nuclei at resting and chromosome at mitotic prophase and metaphase were described and classified according to Levan *et al* (1964) and Tanaka (1971, 1980).

### Results

Chromosome numbers of the 14 species counted in this study were listed in Table 1 and their karyotypes were described as follows.

---

\*Contribution from the Hiroshima Botanical Garden No.97

1)The Hiroshima Botanical Garden

2)Botanical Garden, Technical Center, Hiroshima University

Bulletin of the Hiroshima Botanical Garden, No.30 : 31-50, 2012

Table 1. Chromosome numbers of 14 taxa of subtribe Stanhopeinae studied

Species	HBG* accession number	Chromosome numbers		References
		Present count (2n)	Previous count (2n)	
<i>Acineta</i>				
<i>barkeri</i> (Bateman) Lindl.	1404	40		
<i>superba</i> (Kunth) Rchb.f.	638	40	40,42	Daker & Jones
<i>Cirrhaea</i>				
<i>loddigesii</i> Lindl.	607	40		
<i>Coeliopsis</i>				
<i>hyacinthosma</i> Rchb.f.	647	38		
<i>Gongora</i>				
<i>armeniaca</i> (Lindl.) Rchb.f.	563	38		
<i>truncata</i> Lindl.	602	38	ca.38	Daker & Jones
<i>Kegeliella</i>				
<i>atropilosa</i> L.O.Williams & A.H.Heller	2699	40		
<i>Paphinia</i>				
<i>grandiflora</i> Barb.Rodr.	2220	40		
<i>Peristeria</i>				
<i>elata</i> Hook.	621	40	40	Daker & Jones
<i>Polycycnis</i>				
<i>barbata</i> (Lindl.) Rchb.f.	730	40		
<i>Schlimia</i>				
<i>trifida</i> Rchb.f.	2819	40		
<i>Stanhopea</i>				
<i>cirrhatta</i> Lindl.	2272	40		
<i>guttulata</i> Lindl.	604	40		
<i>pulla</i> Rchb.f.	668	40		

\* Hiroshima Botanical Garden.

***Acineta barkeri* (Bateman) Lindl., HBG1404, Table 1 and 2, Fig. 1.**

Chromosome number: 2n=40

Resting stage (Fig. 1A): complex chromocenter type

Mitotic prophase (Fig. 1B): interstitial type

Mitotic metaphase (Table 2, Fig. 1C and D): 2n=40 chromosome complement exhibited a gradual decrease in length from 3.0µm to 1.3µm and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos. 39 and 40) had the nucleolus organizing region near the centromere as wide heterochromatic gap, which were similar to the description by Daker and Jones (1969) for *Stanhopea* spp.

***Acineta superba* (Kunth)Rchb., HBG638, Table 1 and 3, Fig. 2.**

Chromosome number: 2n=40

Resting stage (Fig. 2A): complex chromocenter type

Mitotic prophase (Fig. 2B): interstitial type

Mitotic metaphase (Table 3, Fig. 2C and D): 2n=40 chromosome complement exhibited a gradual decrease in length from 2.7µm to 1.3µm and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos.35 and 36) were similar to those of *Acineta barkeri* described above.

***Cirrhaea loddigesii* Lindl., HBG607, Table 1 and 4, Fig. 3.**

Chromosome number: 2n=40

Resting stage (Fig. 3A): simple chromocenter type

Mitotic prophase (Fig. 3B): intermediate between proximal type and interstitial type

Mitotic metaphase (Table 4, Fig. 3C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $2.4\mu\text{m}$  to  $1.2\mu\text{m}$  and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos. 39 and 40) were similar to those of *Acineta barkeri*.

***Coeliopsis hyacinthosma* Rchb.f., HBG647, Table 1 and 5, Fig. 4.**

Chromosome number:  $2n=38$

Resting stage (Fig. 4A): complex chromocenter type

Mitotic prophase (Fig. 4B): interstitial type

Mitotic metaphase (Table 5, Fig. 4C and D):  $2n=38$  chromosome complement exhibited a gradual decrease in length from  $2.3\mu\text{m}$  to  $0.6\mu\text{m}$  and a symmetric karyotype with respect to their form. The centromere was not observed in three chromosomes (Nos. 36, 37 and 38).

***Gongora armeniaca* Rchb.f., HBG563, Table 1 and 6, Fig. 5.**

Chromosome number:  $2n=38$

Resting stage (Fig. 5A): complex chromocenter type

Mitotic prophase (Fig. 5B): interstitial type

Mitotic metaphase (Table 6, Fig. 5C and D):  $2n=38$  chromosome complement exhibited a gradual decrease in length from  $2.9\mu\text{m}$  to  $1.4\mu\text{m}$  and a symmetric karyotype with respect to their form. The satellite chromosome (Nos. 30) was similar to those of *Acineta barkeri*.

***Gongora truncata* Lindl., HBG602, Table 1 and 7, Fig. 6.**

Chromosome number:  $2n=38$

Resting stage (Fig. 6A): complex chromocenter type

Mitotic prophase (Fig. 6B): interstitial type

Mitotic metaphase (Table 7, Fig. 6C and D):  $2n=38$  chromosome complement exhibited a gradual decrease in length from  $2.5\mu\text{m}$  to  $1.1\mu\text{m}$  and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos. 31 and 32) were similar to those of *Acineta barkeri*.

***Kegeliella atropilosa* L.O. Williams, HBG2699, Table 1 and 8, Fig. 7.**

Chromosome number:  $2n=40$

Resting stage (Fig. 7A): complex chromocenter type

Mitotic prophase (Fig. 7B): interstitial type

Mitotic metaphase (Table 8, Fig. 7C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $4.0\mu\text{m}$  to  $1.4\mu\text{m}$  and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos. 23 and 24) were similar to those of *Acineta barkeri*.

***Paphinia grandiflora* Barb. Rodrig., HBG2220, Table 1 and 9, Fig. 8.**

Chromosome number:  $2n=40$

Resting stage (Fig. 8A): complex chromocenter type

Mitotic prophase (Fig. 8B): interstitial type

Mitotic metaphase (Table 9, Fig. 8C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $2.5\mu\text{m}$  to  $1.2\mu\text{m}$  and a symmetric karyotype with respect to their form. The centromere was not observed in a chromosome (Nos. 26).

***Peristeria elata* Hook. , HBG621, Table 1 and 10, Fig. 9.**

Chromosome number:  $2n=40$

Resting stage (Fig. 9A): complex chromocenter type

Mitotic prophase (Fig. 9B): interstitial type

Mitotic metaphase (Table 10, Fig. 9C and D):  $2n=38$  chromosome complement exhibited a gradual decrease in length from  $2.9\mu\text{m}$  to  $1.2\mu\text{m}$  and a symmetric karyotype with respect to their form.

***Polycynis barbata* (Lindl.) Rchb.f., HBG730, Table 1 and 11, Fig. 10.**

Chromosome number:  $2n=40$

Resting stage (Fig. 10A): complex chromocenter type

Mitotic prophase (Fig. 10B): interstitial type

Mitotic metaphase (Table 11, Fig. 10C and D):  $2n=40$  chromosome complement formed a bimodality in the chromosome alignment in length with a group of chromosomes (Nos. 1-36) ranging from  $2.2-1.0\mu\text{m}$  and with the other group of chromosomes (Nos. 37-40) ranging from  $0.5-0.4\mu\text{m}$ . The centromere was not observed in five chromosomes (Nos. 36-40), though the complement exhibited a symmetric karyotype with respect to their form.

***Schlimia trifida* Rchb.f., HBG2819, Table 1 and 12, Fig. 11.**

Chromosome number:  $2n=40$

Resting stage (Fig. 11A): complex chromocenter type

Mitotic prophase (Fig. 11B): interstitial type

Mitotic metaphase (Table 12, Fig. 11C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $2.5\mu\text{m}$  to  $1.0\mu\text{m}$  and an intermediate karyotype between the symmetric and asymmetric karyotypes with respect to their form. *Schlimia trifida* had eight subterminal chromosomes (Nos. 4-8 and 21-23) which were not found in other species. Thus, the karyotype of this species was clearly different from those of other species. Two satellite chromosomes (Nos. 37 and 38) were similar to those of *Acineta barkeri*.

***Stanhopea cirrhata* Lindl. HBG2272, Table 1 and 13, Fig. 12.**

Chromosome number:  $2n=40$

Resting stage (Fig. 12A): complex chromocenter type

Mitotic prophase (Fig. 12B): interstitial type

Mitotic metaphase (Table 13, Fig. 12C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $1.4\mu\text{m}$  to  $0.7\mu\text{m}$  and a symmetric karyotype with respect to their form. The centromere was not observed in 13 chromosomes (Nos. 10, 20, 23 and 31-40).

***Stanhopea guttulata* Lindl. HBG604, Table 1 and 14, Fig. 13.**

Chromosome number:  $2n=40$

Resting stage (Fig. 13A): complex chromocenter type

Mitotic prophase (Fig. 13B): interstitial type

Mitotic metaphase (Table 14, Fig. 13C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $2.3\mu\text{m}$  to  $0.8\mu\text{m}$  and a symmetric karyotype with respect to their form. Two satellite chromosomes (Nos. 5 and 6) were similar to those of *Acineta barkeri*.

***Stanhopea pulla* Rchb.f. HBG668, Table 1 and 15, Fig. 14.**

Chromosome number:  $2n=40$

Resting stage (Fig. 14A): complex chromocenter type

Mitotic prophase (Fig. 14B): interstitial type

Mitotic metaphase (Table 15, Fig. 14C and D):  $2n=40$  chromosome complement exhibited a gradual decrease in length from  $1.5\mu\text{m}$  to  $0.8\mu\text{m}$  and a symmetric karyotype with respect to their form. The centromere was not observed in 17 chromosomes (Nos. 10, 13, 14, 19, 20, 23, 26-28, and 33-40).

### Discussion and conclusion

Chromosome numbers of 11 species;  $2n=38$  in *Coeliopsis hyacinthosma* and *Gongora armeniaca*,  $2n=40$  in *Acineta barkeri*, *Cirrhaea loddigesii*, *Kegeliella atropilosa*, *Paphinia grandiflora*, *Polycycnis barbata*, *Schlimia trifida*, *Stanhopea cirrhata*, *S. guttulata* and *S. pulla* were revealed in this observation for the first time. Chromosome numbers of  $2n=40$  and  $42$  in *Acineta superba* and  $2n=ca.38$  in *Gongora truncata* reported by Daker and Jones (1969) were verified to  $2n=40$  and  $2n=38$ , respectively. Thus, the subtribe Stanhopeinae included two different chromosome numbers of  $2n=38$  (three species in two genera) and  $2n=40$  (11 species in eight genera).

The chromosome complements of most species studied showed commonly the complex chromocenter type at resting stage, the interstitial type of chromatin condensation at mitotic prophase and the gradual and the symmetric karyotypes at mitotic metaphase. In contrast, that of *Cirrhaea loddegesii* showed the simple chromocenter type at resting stage and the intermediate type between the proximal type and the interstitial type at mitotic prophase, that of *Polycycnis barbata* showed the bimodal karyotype including four small chromosomes, and that of *Schlimia trifida* showed the intermediate karyotype between the symmetric and the asymmetric karyotypes with respect of eight subterminal chromosomes. Satellite chromosome was observed in eight species of six genera. This is characterized by wide heterochromatic region near the centromere and somewhat difficult to be recognized as mentioned by Daker and Jones (1969), so it may have been missed in some species examined in this study.

Chromosome morphologies through mitotic cycle were varied among 14 taxa and it was suggested karyomorphologically that three genera of *Cirrhaea*, *Polycycnis* and *Schlimia* may not be closely related among the members of the subtribe Stanhopeinae.

### References

- Aoyama, M. 1989. Karyomorphological studies in *Cymbidium* and its allied genera, Orchidaceae. Bull. Hiroshima Bot. Gard. 11: 1-121.
- Daker, M.G. & Jones, K. 1969. The chromosomes of Orchids: V Stanhopeinae Benth. (Gongorinae auct.). Kew Bull. 24: 457-459.
- Dressler, R.L. 1993. Phylogeny and classification of the Orchid family. 314pp. Dioscorides Press. Portland, Oregon.
- Hamatani, S. 2011. Characterization of *Lachenalia*, the Liliaceae based on karyomorphological, molecular phylogenetical and molecular cytogenetical studies. Bull. Hiroshima Bot. Gard. 29: 1-90.
- Hamatani, S. & Aoyama, M. 2012. Karyomorphological studies of six species of subtribe Catasetinae, Orchidaceae. Bull. Hiroshima Bot. Gard. 30: 15-30.
- Hashimoto, K. 1981. Chromosome count in *Dendrobium* 1. 87 species. Bull. Hiroshima Bot. Gard. 4: 63-80.
- Ishida, G. 1990. Karyomorphological studies in *Calanthe*, Orchidaceae. Bull. Hiroshima Bot. Gard. 12: 1-69.
- Ishida, G. 2001. Karyomorphological observations on some Aroids cultivated in the Hiroshima Botanical Garden 1. *Alocasia*. Bull. Hiroshima Bot. Gard. 20: 1-33.
- Karasawa, K. 1979. Karyomorphological studies in *Paphiopedilum*, Orchidaceae. Bull. Hiroshima Bot. Gard. 2: 1-149.
- Levan, A., Fredga, K. and Sandberg, A. 1964. Nomenclature for centromeric position on chromosomes. Hereditas 52: 201-220.
- Sera, T. & Karasawa, K. 1984. Karyomorphological studies on 22 species of *Saintpaulia*, Gesneriaceae. Bull. Hiroshima

Bot. Gard. 7: 1-30.

Tanaka, R. 1971. Types of resting nuclei of Orchidaceae. Bot. Mag. Tokyo 84: 118-122.

Tanaka, R. 1980. The karyotype (in Japanese). Kihara, H. (ed.) Plant Genetics I, pp.335-358. Shokabo Co., Tokyo.

## ラン科スタンホペア亜族14種における染色体観察

世羅徹哉<sup>1)</sup>・青山幹男<sup>2)</sup>・石田源次郎<sup>1)</sup>

### 要 約

ラン科, マクシラリア族のスタンホペア亜族に属する 10 属 14 種において, アセトオルセイン押しつぶし法による染色体の観察を行った. その結果, 9 属 11 種の染色体数を初めて明らかにし, 3 属 3 種の染色体数については, 以前の報告を確認した. 14 種の核型は互いによく似ていたが, 静止期核ではキルハエア属が, 体細胞分裂中期ではポリキクニス属とスクリミア属が他の属とは異なる型を示した. 従って, これら 3 属がスタンホペア亜族の他の属と, 核形態学的に類縁関係が遠い可能性のあることが示唆された.

---

1) 広島市植物公園

2) 広島大学技術センター附属植物園

Table 3. Measurements of somatic chromosomes at mitotic metaphase in *Acineta superba*, HBG638, 2n=40

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.1+1.6=2.7	3.2	1.45	m
2	1.1+1.5=2.6	3.1	1.36	m
3	1.2+1.4=2.6	3.1	1.17	m
4	1.2+1.4=2.6	3.1	1.17	m
5	1.1+1.5=2.6	3.1	1.36	m
6	1.2+1.4=2.6	3.1	1.17	m
7	0.9+1.6=2.5	3.0	1.78	sm
8	0.9+1.6=2.5	3.0	1.78	sm
9	1.0+1.5=2.5	3.0	1.50	m
10	1.1+1.4=2.5	3.0	1.27	m
11	1.1+1.4=2.5	3.0	1.27	m
12	1.2+1.3=2.5	3.0	1.08	m
13	1.1+1.3=2.4	2.9	1.18	m
14	1.1+1.3=2.4	2.9	1.18	m
15	1.1+1.2=2.3	2.7	1.09	m
16	1.0+1.3=2.3	2.7	1.30	m
17	1.0+1.2=2.2	2.6	1.20	m
18	0.9+1.3=2.2	2.6	1.44	m
19	1.0+1.1=2.1	2.5	1.10	m
20	1.0+1.1=2.1	2.5	1.10	m
21	0.9+1.2=2.1	2.5	1.33	m
22	0.9+1.1=2.0	2.4	1.22	m
23	0.7+1.3=2.0	2.4	1.86	sm
24	0.7+1.3=2.0	2.4	1.86	sm
25	0.9+1.1=2.0	2.4	1.22	m
26	0.9+1.1=2.0	2.4	1.22	m
27	0.8+1.1=1.9	2.3	1.38	m
28	0.8+1.0=1.8	2.1	1.25	m
29	0.9+0.9=1.8	2.1	1.00	M
30	0.8+1.0=1.8	2.1	1.25	m
31	0.8+1.0=1.8	2.1	1.25	m
32	0.8+1.0=1.8	2.1	1.25	m
33	0.7+1.1=1.8	2.1	1.57	m
34	0.7+1.0=1.7	2.0	1.43	m
35*	0.7+0.9=1.6	1.9	1.29	m
36*	0.6+0.8=1.4	1.7	1.33	m
37	0.6+0.9=1.5	1.8	1.50	m
38	0.6+0.8=1.4	1.7	1.33	m
39	0.6+0.8=1.4	1.8	1.33	m
40	0.6+0.7=1.3	1.7	1.33	m

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 2. Measurements of somatic chromosomes at mitotic metaphase in *Acineta barkeri*, HBG1404, 2n=40

Chromosome	Length ( $\mu\text{m}$ )	Relative length	Arm ratio	Form
1	1.4+1.6=3.0	3.6	1.14	m
2	1.3+1.7=3.0	3.6	1.31	m
3	1.3+1.6=2.9	3.5	1.23	m
4	1.3+1.5=2.8	3.4	1.15	m
5	1.2+1.4=2.6	3.2	1.17	m
6	1.1+1.5=2.6	3.2	1.36	m
7	1.1+1.4=2.5	3.0	1.27	m
8	1.1+1.4=2.5	3.0	1.27	m
9	1.1+1.3=2.4	2.9	1.18	m
10	1.2+1.2=2.4	2.9	1.00	M
11	1.2+1.2=2.4	2.9	1.00	M
12	1.0+1.3=2.3	2.8	1.30	m
13	0.8+1.5=2.3	2.8	1.88	sm
14	0.8+1.5=2.3	2.8	1.88	sm
15	1.1+1.2=2.3	2.8	1.09	m
16	1.0+1.2=2.2	2.7	1.20	m
17	1.0+1.1=2.1	2.6	1.10	m
18	1.0+1.1=2.1	2.6	1.10	m
19	0.7+1.4=2.1	2.6	2.00	sm
20	0.7+1.4=2.1	2.6	2.00	sm
21	0.9+1.1=2.0	2.4	1.22	m
22	0.9+1.1=2.0	2.4	1.22	m
23	0.8+1.1=1.9	2.3	1.38	m
24	0.9+1.0=1.9	2.3	1.11	m
25	0.7+1.2=1.9	2.3	1.71	sm
26	0.5+1.3=1.8	2.2	2.60	sm
27	0.7+1.1=1.8	2.2	1.57	m
28	0.7+1.1=1.8	2.2	1.57	m
29	0.8+0.9=1.7	2.1	1.13	m
30	0.7+1.0=1.7	2.1	1.43	m
31	0.6+1.1=1.7	2.1	1.83	sm
32	0.8+0.9=1.7	2.1	1.13	m
33	0.8+0.8=1.6	1.9	1.00	M
34	0.8+0.8=1.6	1.9	1.00	M
35	0.7+0.8=1.5	1.8	1.14	m
36	0.6+0.8=1.4	1.7	1.33	m
37	0.4+1.0=1.4	1.7	2.50	sm
38	0.4+0.9=1.3	1.6	2.25	sm
39*	0.4+1.0=1.4	1.7	2.50	sm
40*	0.4+0.9=1.3	1.6	2.25	sm

\* Satellite chromosome (chromosome with wide heterochromatic region).



Table 4. Measurements of somatic chromosomes at mitotic metaphase in *Cirrhaea loddigesii*, HBG607, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.1+1.3=2.4	3.3	1.18	m
2	1.0+1.4=2.4	3.3	1.40	m
3	1.0+1.3=2.3	3.1	1.30	m
4	1.0+1.3=2.3	3.1	1.30	m
5	1.1+1.1=2.2	3.0	1.00	M
6	1.0+1.2=2.2	3.0	1.20	m
7	1.0+1.2=2.2	3.0	1.20	m
8	0.9+1.3=2.2	3.0	1.44	m
9	0.8+1.4=2.2	3.0	1.75	sm
10	0.8+1.4=2.2	3.0	1.75	sm
11	0.7+1.4=2.1	2.9	2.00	sm
12	0.7+1.4=2.1	2.9	2.00	sm
13	1.0+1.1=2.1	2.9	1.10	m
14	1.0+1.1=2.1	2.9	1.10	m
15	0.8+1.1=1.9	2.6	1.38	m
16	0.8+1.1=1.9	2.6	1.38	m
17	0.7+1.2=1.9	2.6	1.71	sm
18	0.7+1.2=1.9	2.6	1.71	sm
19	0.9+0.9=1.8	2.5	1.00	M
20	0.8+1.0=1.8	2.5	1.25	m
21	0.8+0.9=1.7	2.3	1.13	m
22	0.8+0.9=1.7	2.3	1.13	m
23	0.6+1.1=1.7	2.3	1.83	sm
24	0.6+1.1=1.7	2.3	1.83	sm
25	0.6+1.1=1.7	2.3	1.83	sm
26	0.6+1.1=1.7	2.3	1.83	sm
27	0.6+1.1=1.7	2.3	1.83	sm
28	0.6+1.1=1.7	2.3	1.83	sm
29	0.6+1.1=1.7	2.3	1.83	sm
30	0.6+1.1=1.7	2.3	1.83	sm
31	0.5+1.1=1.6	2.2	2.20	sm
32	0.5+1.1=1.6	2.2	2.20	sm
33	0.5+1.0=1.5	2.0	2.00	sm
34	0.5+1.0=1.5	2.0	2.00	sm
35	0.5+0.9=1.4	1.9	1.80	sm
36	0.5+0.9=1.4	1.9	1.80	sm
37	0.6+0.8=1.4	1.9	1.33	m
38	0.6+0.8=1.4	1.9	1.33	m
39*	0.4+0.8=1.2	1.6	2.00	sm
40*	0.4+0.8=1.2	1.6	2.00	sm

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 5. Measurements of somatic chromosomes at mitotic metaphase in *Coelopsis hyacinthosma*, HBG647, 2n=38

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.0+1.3=2.3	3.7	1.30	m
2	1.0+1.2=2.2	3.5	1.20	m
3	0.7+1.5=2.2	3.5	2.14	sm
4	0.7+1.5=2.2	3.5	2.14	sm
5	1.0+1.1=2.1	3.3	1.10	m
6	0.8+1.3=2.1	3.3	1.63	m
7	0.7+1.4=2.1	3.3	2.00	sm
8	0.7+1.3=2.0	3.2	1.86	sm
9	0.6+1.4=2.0	3.2	2.33	sm
10	0.6+1.4=2.0	3.2	2.33	sm
11	0.8+1.1=1.9	3.0	1.38	m
12	0.8+1.1=1.9	3.0	1.38	m
13	0.8+1.1=1.9	3.0	1.38	m
14	0.7+1.2=1.9	3.0	1.71	sm
15	0.6+1.3=1.9	3.0	2.17	sm
16	0.6+1.2=1.8	2.9	2.00	sm
17	0.6+1.2=1.8	2.9	2.00	sm
18	0.6+1.2=1.8	2.9	2.00	sm
19	0.8+1.0=1.8	2.9	1.25	m
20	0.8+1.0=1.8	2.9	1.25	m
21	0.6+1.1=1.7	2.7	1.83	sm
22	0.6+1.1=1.7	2.7	1.83	sm
23	0.8+0.8=1.6	2.5	1.00	M
24	0.6+1.0=1.6	2.5	1.67	m
25	0.5+1.0=1.5	2.4	2.00	sm
26	0.5+1.0=1.5	2.4	2.00	sm
27	0.6+0.8=1.4	2.2	1.33	m
28	0.6+0.8=1.4	2.2	1.33	m
29	0.6+0.8=1.4	2.2	1.33	m
30	0.6+0.8=1.4	2.2	1.33	m
31	0.5+0.8=1.3	2.1	1.60	m
32	0.4+0.8=1.2	1.9	2.00	sm
33	0.5+0.7=1.2	1.9	1.40	m
34	0.5+0.6=1.1	1.7	1.20	m
35	0.4+0.7=1.1	1.7	1.75	sm
36*	0.9	1.4		
37*	0.7	1.1		
38*	0.6	1.0		

\* The centromere was not observed.

Table 7. Measurements of somatic chromosomes at mitotic metaphase in *Gongora truncata*, HBG602, 2n=38

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.1+1.4=2.5	3.9	1.27	m
2	1.2+1.2=2.4	3.7	1.00	M
3	0.9+1.6=2.5	3.9	1.78	sm
4	0.9+1.6=2.5	3.9	1.78	sm
5	0.7+1.6=2.3	3.6	2.29	sm
6	0.7+1.6=2.3	3.6	2.29	sm
7	1.0+1.2=2.2	3.4	1.20	m
8	1.0+1.2=2.2	3.4	1.20	m
9	0.7+1.3=2.0	3.1	1.86	sm
10	0.7+1.3=2.0	3.1	1.86	sm
11	0.9+1.1=2.0	3.1	1.22	m
12	0.9+1.1=2.0	3.1	1.22	m
13	0.9+1.1=2.0	3.1	1.22	m
14	0.8+1.2=2.0	3.1	1.50	m
15	0.8+1.2=2.0	3.1	1.50	m
16	0.8+1.1=1.9	3.0	1.38	m
17	0.6+1.0=1.6	2.5	1.67	m
18	0.6+1.0=1.6	2.5	1.67	m
19	0.7+1.0=1.7	2.6	1.43	m
20	0.7+1.0=1.7	2.6	1.43	m
21	0.6+0.8=1.4	2.2	1.33	m
22	0.6+0.8=1.4	2.2	1.33	m
23	0.6+0.8=1.4	2.2	1.33	m
24	0.6+0.8=1.4	2.2	1.33	m
25	0.4+1.0=1.4	2.2	2.50	sm
26	0.4+1.0=1.4	2.2	2.50	sm
27	0.6+0.8=1.4	2.2	1.33	m
28	0.6+0.8=1.4	2.2	1.33	m
29	0.6+0.6=1.2	1.9	1.00	M
30	0.6+0.6=1.2	1.9	1.00	M
31*	0.6+0.6=1.2	1.9	1.00	M
32*	0.6+0.6=1.2	1.9	1.00	M
33	0.3+0.9=1.2	1.9	3.00	sm
34	0.3+0.9=1.2	1.9	3.00	sm
35	0.5+0.6=1.1	1.7	1.20	m
36	0.5+0.6=1.1	1.7	1.20	m
37	0.5+0.6=1.1	1.7	1.20	m
38	0.5+0.6=1.1	1.7	1.20	m

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 6. Measurements of somatic chromosomes at mitotic metaphase in *Gongora armeniaca*, HBG563, 2n=38

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.2+1.7=2.9	3.7	1.42	m
2	1.2+1.7=2.9	3.7	1.42	m
3	1.1+1.6=2.7	3.4	1.45	m
4	1.0+1.7=2.7	3.4	1.70	m
5	1.1+1.5=2.6	3.3	1.36	m
6	1.2+1.4=2.6	3.3	1.17	m
7	0.8+1.8=2.6	3.3	2.25	sm
8	0.9+1.7=2.6	3.3	1.89	sm
9	1.1+1.4=2.5	3.2	1.27	m
10	1.2+1.2=2.4	3.0	1.00	M
11	0.8+1.5=2.3	2.9	1.88	sm
12	0.8+1.5=2.3	2.9	1.88	sm
13	1.1+1.2=2.3	2.9	1.09	m
14	1.1+1.2=2.3	2.9	1.09	m
15	0.8+1.5=2.3	2.9	1.88	sm
16	0.8+1.4=2.2	2.8	1.75	sm
17	0.9+1.2=2.1	2.7	1.33	m
18	0.9+1.2=2.1	2.7	1.33	m
19	0.7+1.3=2.0	2.5	1.86	sm
20	0.7+1.4=2.1	2.7	2.00	sm
21	0.7+1.3=2.0	2.5	1.86	sm
22	0.7+1.3=2.0	2.5	1.86	sm
23	0.8+1.2=2.0	2.5	1.50	m
24	0.8+1.1=1.9	2.4	1.38	m
25	0.7+1.2=1.9	2.4	1.71	sm
26	0.7+1.2=1.9	2.4	1.71	sm
27	0.7+1.2=1.9	2.4	1.71	sm
28	0.6+1.1=1.7	2.2	1.83	sm
29	0.8+0.8=1.6	2.0	1.00	M
30*	0.8+0.8=1.6	2.0	1.00	M
31	0.8+0.8=1.6	2.0	1.00	M
32	0.7+0.9=1.6	2.0	1.29	m
33	0.7+0.8=1.5	1.9	1.14	m
34	0.6+0.9=1.5	1.9	1.50	m
35	0.6+0.8=1.4	1.8	1.33	m
36	0.6+0.8=1.4	1.8	1.33	m
37	0.6+0.8=1.4	1.8	1.33	m
38	0.6+0.8=1.4	1.8	1.33	m

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 8. Measurements of somatic chromosomes at mitotic metaphase in *Kegeliella atropilosa*, HBG2699, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.9+2.1=4.0	4.4	1.11	m
2	1.6+1.7=3.3	3.6	1.06	m
3	1.1+2.1=3.2	3.5	1.91	sm
4	1.0+2.1=3.1	3.4	2.10	sm
5	1.5+1.6=3.1	3.4	1.07	m
6	1.2+1.8=3.0	3.3	1.50	m
7	1.2+1.7=2.9	3.2	1.42	m
8	1.3+1.4=2.7	3.0	1.08	m
9	0.7+2.0=2.7	3.0	2.86	sm
10	0.9+1.7=2.6	2.9	1.89	sm
11	0.8+1.8=2.6	2.9	2.25	sm
12	0.8+1.8=2.6	2.9	2.25	sm
13	1.2+1.3=2.5	2.8	1.08	m
14	1.1+1.4=2.5	2.8	1.27	m
15	1.1+1.3=2.4	2.6	1.18	m
16	1.1+1.3=2.4	2.6	1.18	m
17	0.8+1.5=2.3	2.5	1.88	sm
18	0.6+1.6=2.2	2.4	2.67	sm
19	0.6+1.6=2.2	2.4	2.67	sm
20	0.6+1.6=2.2	2.4	2.67	sm
21	1.0+1.2=2.2	2.4	1.20	m
22	1.0+1.2=2.2	2.4	1.20	m
23*	0.8+1.4=2.2	2.4	1.75	sm
24*	0.7+1.5=2.2	2.4	2.14	sm
25	0.8+1.3=2.1	2.3	1.63	m
26	0.8+1.3=2.1	2.3	1.63	m
27	0.8+1.2=2.0	2.2	1.50	m
28	0.8+1.3=2.1	2.3	1.63	m
29	0.8+1.0=1.8	2.0	1.25	m
30	0.8+1.0=1.8	2.0	1.25	m
31	0.7+1.0=1.7	1.9	1.43	m
32	0.8+0.9=1.7	1.9	1.13	m
33	0.8+0.9=1.7	1.9	1.13	m
34	0.7+1.0=1.7	1.9	1.43	m
35	0.7+0.9=1.6	1.8	1.29	m
36	0.7+0.8=1.5	1.7	1.14	m
37	0.6+0.9=1.5	1.7	1.50	m
38	0.6+0.8=1.4	1.5	1.33	m
39	0.6+0.8=1.4	1.5	1.33	m
40	0.6+0.8=1.4	1.5	1.33	m

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 9. Measurements of somatic chromosomes at mitotic metaphase in *Paphinia grandiflora*, HBG2220, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.0+1.5=2.5	3.5	1.50	m
2	1.0+1.5=2.5	3.5	1.50	m
3	0.8+1.7=2.5	3.5	2.13	sm
4	0.8+1.6=2.4	3.3	2.00	sm
5	0.6+1.7=2.3	3.2	2.83	sm
6	0.5+1.8=2.3	3.2	3.60	t
7	0.9+1.3=2.2	3.1	1.44	m
8	0.9+1.3=2.2	3.1	1.44	m
9	0.9+1.2=2.1	2.9	1.33	m
10	0.9+1.2=2.1	2.9	1.33	m
11	0.6+1.5=2.1	2.9	2.50	sm
12	0.6+1.4=2.0	2.8	2.33	sm
13	0.8+1.1=1.9	2.6	1.38	m
14	0.8+1.1=1.9	2.6	1.38	m
15	0.6+1.3=1.9	2.6	2.17	sm
16	0.6+1.3=1.9	2.6	2.17	sm
17	0.6+1.3=1.9	2.6	2.17	sm
18	0.6+1.3=1.9	2.6	2.17	sm
19	0.7+1.2=1.9	2.6	1.71	sm
20	0.5+1.3=1.8	2.5	2.60	sm
21	0.5+1.3=1.8	2.5	2.60	sm
22	0.5+1.3=1.8	2.5	2.60	sm
23	0.7+1.1=1.8	2.5	1.57	m
24	0.7+1.1=1.8	2.5	1.57	m
25	0.5+1.2=1.7	2.4	2.40	sm
26*	1.6	2.2		
27	0.7+0.9=1.6	2.2	1.29	m
28	0.6+1.0=1.6	2.2	1.67	m
29	0.6+0.9=1.5	2.1	1.50	m
30	0.6+0.9=1.5	2.1	1.50	m
31	0.6+0.9=1.5	2.1	1.50	m
32	0.7+0.7=1.4	1.9	1.00	M
33	0.5+0.8=1.3	1.8	1.60	m
34	0.6+0.7=1.3	1.8	1.17	m
35	0.6+0.7=1.3	1.8	1.17	m
36	0.6+0.7=1.3	1.8	1.17	m
37	0.4+0.8=1.2	1.7	2.00	sm
38	0.4+0.8=1.2	1.7	2.00	sm
39	0.6+0.6=1.2	1.7	1.00	M
40	0.5+0.7=1.2	1.7	1.40	m

\* The centromere was not observed.

Table 11. Measurements of somatic chromosomes at mitotic metaphase in *Polycycnis barbata*, HBG730, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.0+1.2=2.2	3.9	1.20	m
2	0.9+1.2=2.1	3.7	1.33	m
3	0.9+1.1=2.0	3.6	1.22	m
4	0.7+1.2=1.9	3.4	1.71	sm
5	0.7+1.2=1.9	3.4	1.71	sm
6	0.8+1.0=1.8	3.2	1.25	m
7	0.8+0.9=1.7	3.0	1.13	m
8	0.8+0.9=1.7	3.0	1.13	m
9	0.8+0.9=1.7	3.0	1.13	m
10	0.8+0.9=1.7	3.0	1.13	m
11	0.8+0.9=1.7	3.0	1.13	m
12	0.7+1.0=1.7	3.0	1.43	m
13	0.6+1.1=1.7	3.0	1.83	sm
14	0.6+1.1=1.7	3.0	1.83	sm
15	0.7+0.9=1.6	2.8	1.29	m
16	0.6+1.0=1.6	2.8	1.67	m
17	0.7+0.8=1.5	2.7	1.14	m
18	0.7+0.8=1.5	2.7	1.14	m
19	0.6+0.9=1.5	2.7	1.50	m
20	0.6+0.9=1.5	2.7	1.50	m
21	0.5+0.9=1.4	2.5	1.80	sm
22	0.5+0.9=1.4	2.5	1.80	sm
23	0.5+0.8=1.3	2.3	1.60	m
24	0.5+0.8=1.3	2.3	1.60	m
25	0.5+0.8=1.3	2.3	1.60	m
26	0.5+0.8=1.3	2.3	1.60	m
27	0.6+0.7=1.3	2.3	1.17	m
28	0.6+0.7=1.3	2.3	1.17	m
29	0.6+0.7=1.3	2.3	1.17	m
30	0.6+0.6=1.2	2.1	1.00	M
31	0.6+0.6=1.2	2.1	1.00	M
32	0.5+0.7=1.2	2.1	1.40	m
33	0.5+0.5=1.0	1.8	1.00	M
34	0.5+0.5=1.0	1.8	1.00	M
35	0.4+0.6=1.0	1.8	1.50	m
36*	1.0	1.8		
37*	0.5	0.9		
38*	0.5	0.9		
39*	0.4	0.7		
40*	0.4	0.7		

\* The centromere was not observed.

Table 10. Measurements of somatic chromosomes at mitotic metaphase in *Peristeria elata*, HBG621, 2n=40

Chromosome	Length(µm)	Relative length	Arm ratio	Form
1	1.3+1.6=2.9	3.8	1.23	m
2	1.2+1.6=2.8	3.7	1.33	m
3	1.0+1.4=2.4	3.2	1.40	m
4	1.0+1.3=2.3	3.1	1.30	m
5	1.1+1.2=2.3	3.1	1.09	m
6	1.0+1.3=2.3	3.1	1.30	m
7	0.9+1.4=2.3	3.1	1.56	m
8	0.9+1.4=2.3	3.1	1.56	m
9	0.8+1.4=2.2	2.9	1.75	sm
10	0.8+1.4=2.2	2.9	1.75	sm
11	0.9+1.2=2.1	2.8	1.33	m
12	0.8+1.3=2.1	2.8	1.63	m
13	0.7+1.4=2.1	2.8	2.00	sm
14	0.7+1.4=2.1	2.8	2.00	sm
15	0.9+1.1=2.0	2.7	1.22	m
16	0.9+1.1=2.0	2.7	1.22	m
17	0.9+1.1=2.0	2.7	1.22	m
18	0.8+1.1=1.9	2.5	1.38	m
19	0.8+1.1=1.9	2.5	1.38	m
20	0.9+1.0=1.9	2.5	1.11	m
21	0.6+1.3=1.9	2.5	2.17	sm
22	0.6+1.3=1.9	2.5	2.17	sm
23	0.6+1.2=1.8	2.4	2.00	sm
24	0.6+1.2=1.8	2.4	2.00	sm
25	0.7+1.1=1.8	2.4	1.57	m
26	0.7+1.1=1.8	2.4	1.57	m
27	0.8+1.0=1.8	2.4	1.25	m
28	0.7+1.1=1.8	2.4	1.57	m
29	0.6+1.1=1.7	2.3	1.83	sm
30	0.6+1.0=1.6	2.1	1.67	m
31	0.6+0.9=1.5	2.0	1.50	m
32	0.6+0.9=1.5	2.0	1.50	m
33	0.6+0.8=1.4	1.9	1.33	m
34	0.6+0.8=1.4	1.9	1.33	m
35	0.5+0.9=1.4	1.9	1.80	sm
36	0.5+0.9=1.4	1.9	1.80	sm
37	0.6+0.6=1.2	1.6	1.00	M
38	0.5+0.7=1.2	1.6	1.40	m
39	0.5+0.7=1.2	1.6	1.40	m
40	0.5+0.7=1.2	1.6	1.40	m

Table 12. Measurements of somatic chromosomes at mitotic metaphase in *Schlimia trifida*, HBG2819, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	0.8+1.7=2.5	3.6	2.13	sm
2	0.8+1.7=2.5	3.6	2.13	sm
3	0.7+1.8=2.5	3.6	2.57	sm
4	0.6+1.9=2.5	3.6	3.17	st
5	0.6+1.9=2.5	3.6	3.17	st
6	0.6+1.9=2.5	3.6	3.17	st
7	0.5+1.7=2.2	3.2	3.40	st
8	0.5+1.7=2.2	3.2	3.40	st
9	0.6+1.4=2.0	2.9	2.33	sm
10	0.6+1.3=1.9	2.8	2.17	sm
11	0.5+1.4=1.9	2.8	2.80	sm
12	0.5+1.4=1.9	2.8	2.80	sm
13	0.5+1.3=1.8	2.6	2.60	sm
14	0.5+1.3=1.8	2.6	2.60	sm
15	0.7+1.0=1.7	2.5	1.43	m
16	0.7+1.0=1.7	2.5	1.43	m
17	0.5+1.2=1.7	2.5	2.40	sm
18	0.5+1.2=1.7	2.5	2.40	sm
19	0.5+1.2=1.7	2.5	2.40	sm
20	0.5+1.2=1.7	2.5	2.40	sm
21	0.4+1.3=1.7	2.5	3.25	st
22	0.4+1.3=1.7	2.5	3.25	st
23	0.4+1.3=1.7	2.5	3.25	st
24	0.4+1.2=1.6	2.3	3.00	sm
25	0.6+1.0=1.6	2.3	1.67	m
26	0.6+1.0=1.6	2.3	1.67	m
27	0.7+0.8=1.5	2.2	1.14	m
28	0.6+0.9=1.5	2.2	1.50	m
29	0.6+0.9=1.5	2.2	1.50	m
30	0.6+0.9=1.5	2.2	1.50	m
31	0.6+0.8=1.4	2.0	1.33	m
32	0.5+0.9=1.4	2.0	1.80	sm
33	0.5+0.8=1.3	1.9	1.60	m
34	0.5+0.7=1.2	1.7	1.40	m
35	0.5+0.7=1.2	1.7	1.40	m
36	0.5+0.6=1.1	1.6	1.20	m
37*	0.5+0.6=1.1	1.6	1.20	m
38*	0.5+0.6=1.1	1.6	1.20	m
39	0.4+0.6=1.0	1.5	1.50	m
40	0.4+0.6=1.0	1.5	1.50	m

\* Satellite chromosome (chromosome with wide heterochromatic region).

Table 13. Measurements of somatic chromosomes at mitotic metaphase in *Stanhopea cirrhata*, HBG2272, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	0.7+0.7=1.4	3.4	1.00	M
2	0.7+0.7=1.4	3.4	1.00	M
3	0.6+0.8=1.4	3.4	1.33	m
4	0.6+0.8=1.4	3.4	1.33	m
5	0.5+0.9=1.4	3.4	1.80	sm
6	0.5+0.8=1.3	3.2	1.60	m
7	0.5+0.8=1.3	3.2	1.60	m
8	0.4+0.8=1.2	2.9	2.00	sm
9	0.4+0.8=1.2	2.9	2.00	sm
10*	1.2	2.9		
11	0.5+0.6=1.1	2.7	1.20	m
12	0.4+0.7=1.1	2.7	1.75	sm
13	0.4+0.7=1.1	2.7	1.75	sm
14	0.4+0.7=1.1	2.7	1.75	sm
15	0.4+0.6=1.0	2.5	1.50	m
16	0.4+0.6=1.0	2.5	1.50	m
17	0.3+0.7=1.0	2.5	2.33	sm
18	0.5+0.5=1.0	2.5	1.00	M
19	0.4+0.6=1.0	2.5	1.50	m
20*	1.0	2.5		
21	0.4+0.5=0.9	2.2	1.25	m
22	0.4+0.5=0.9	2.2	1.25	m
23*	0.9	2.2		
24	0.4+0.5=0.9	2.2	1.25	m
25	0.4+0.5=0.9	2.2	1.25	m
26	0.4+0.5=0.9	2.2	1.25	m
27	0.4+0.5=0.9	2.2	1.25	m
28	0.4+0.5=0.9	2.2	1.25	m
29	0.4+0.5=0.9	2.2	1.25	m
30	0.4+0.5=0.9	2.2	1.25	m
31*	0.9	2.2		
32*	0.9	2.2		
33*	0.9	2.2		
34*	0.9	2.2		
35*	0.8	2.0		
36*	0.8	2.0		
37*	0.8	2.0		
38*	0.8	2.0		
39*	0.7	1.7		
40*	0.7	1.7		

\* The centromere was not observed.

Table 15. Measurements of somatic chromosomes at mitotic metaphase in *Stanhopea pulla*, HBG668, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	0.6+0.9=1.5	3.4	1.50	m
2	0.7+0.8=1.5	3.4	1.14	m
3	0.6+0.8=1.4	3.2	1.33	m
4	0.6+0.8=1.4	3.2	1.33	m
5	0.6+0.7=1.3	3.0	1.17	m
6	0.6+0.7=1.3	3.0	1.17	m
7	0.5+0.8=1.3	3.0	1.60	m
8	0.5+0.8=1.3	3.0	1.60	m
9	0.5+0.8=1.3	3.0	1.60	m
10*	1.3	3.0		
11	0.4+0.9=1.3	3.0	2.25	sm
12	0.4+0.9=1.3	3.0	2.25	sm
13*	1.2	2.7		
14*	1.2	2.7		
15	0.5+0.7=1.2	2.7	1.40	m
16	0.5+0.7=1.2	2.7	1.40	m
17	0.5+0.6=1.1	2.5	1.20	m
18	0.5+0.6=1.1	2.5	1.20	m
19*	1.1	2.5		
20*	1.1	2.5		
21	0.5+0.6=1.1	2.5	1.20	m
22	0.5+0.6=1.1	2.5	1.20	m
23*	1.1	2.5		
24	0.5+0.5=1.0	2.3	1.00	M
25	0.4+0.6=1.0	2.3	1.50	m
26*	1.0	2.3		
27*	1.0	2.3		
28*	1.0	2.3		
29	0.4+0.5=0.9	2.0	1.25	m
30	0.4+0.5=0.9	2.0	1.25	m
31	0.4+0.5=0.9	2.0	1.25	m
32	0.4+0.5=0.9	2.0	1.25	m
33*	0.9	2.0		
34*	0.9	2.0		
35*	0.9	2.0		
36*	0.8	1.8		
37*	0.8	1.8		
38*	0.8	1.8		
39*	0.8	1.8		
40*	0.8	1.8		

\* The centromere was not observed.

Table 14. Measurements of somatic chromosomes at mitotic metaphase in *Stanhopea guttulata*, HBG604, 2n=40

Chromosome	Length (µm)	Relative length	Arm ratio	Form
1	1.0+1.3=2.3	3.9	1.30	m
2	0.8+1.5=2.3	3.9	1.88	sm
3	0.9+1.2=2.1	3.5	1.33	m
4	0.8+1.2=2.0	3.4	1.50	m
5*	0.6+1.4=2.0	3.4	2.33	sm
6*	0.6+1.4=2.0	3.4	2.33	sm
7	0.9+1.0=1.9	3.2	1.11	m
8	0.9+1.0=1.9	3.2	1.11	m
9	0.8+0.9=1.7	2.9	1.13	m
10	0.8+0.9=1.7	2.9	1.13	m
11	0.6+1.1=1.7	2.9	1.83	sm
12	0.7+1.0=1.7	2.9	1.43	m
13	0.7+0.9=1.6	2.7	1.29	m
14	0.7+0.9=1.6	2.7	1.29	m
15	0.7+0.9=1.6	2.7	1.29	m
16	0.7+0.8=1.5	2.5	1.14	m
17	0.7+0.8=1.5	2.5	1.14	m
18	0.6+0.9=1.5	2.5	1.50	m
19	0.6+0.9=1.5	2.5	1.50	m
20	0.7+0.7=1.4	2.4	1.00	M
21	0.6+0.8=1.4	2.4	1.33	m
22	0.6+0.8=1.4	2.4	1.33	m
23	0.6+0.8=1.4	2.4	1.33	m
24	0.5+0.8=1.3	2.2	1.60	m
25	0.5+0.8=1.3	2.2	1.60	m
26	0.5+0.8=1.3	2.2	1.60	m
27	0.5+0.8=1.3	2.2	1.60	m
28	0.5+0.8=1.3	2.2	1.60	m
29	0.5+0.8=1.3	2.2	1.60	m
30	0.6+0.7=1.3	2.2	1.17	m
31	0.6+0.7=1.3	2.2	1.17	m
32	0.6+0.6=1.2	2	1.00	M
33	0.5+0.7=1.2	2	1.40	m
34	0.5+0.6=1.1	1.8	1.20	m
35	0.5+0.6=1.1	1.8	1.20	m
36	0.5+0.6=1.1	1.8	1.20	m
37	0.4+0.6=1.0	1.7	1.50	m
38	0.4+0.5=0.9	1.5	1.25	m
39	0.4+0.4=0.8	1.3	1.00	M
40	0.4+0.4=0.8	1.3	1.00	M

\* Satellite chromosome (chromosome with wide heterochromatic region).

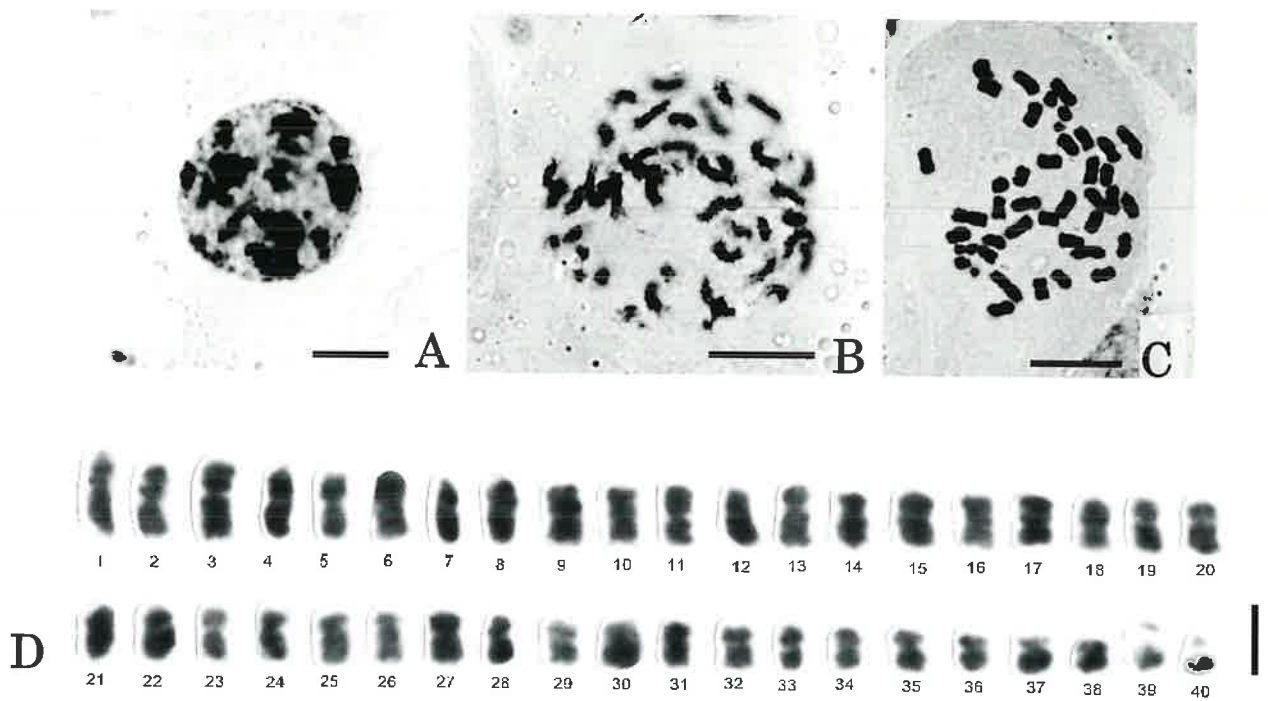


Fig. 1. Chromosomes of *Acineta barkeri*, HBG1404,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

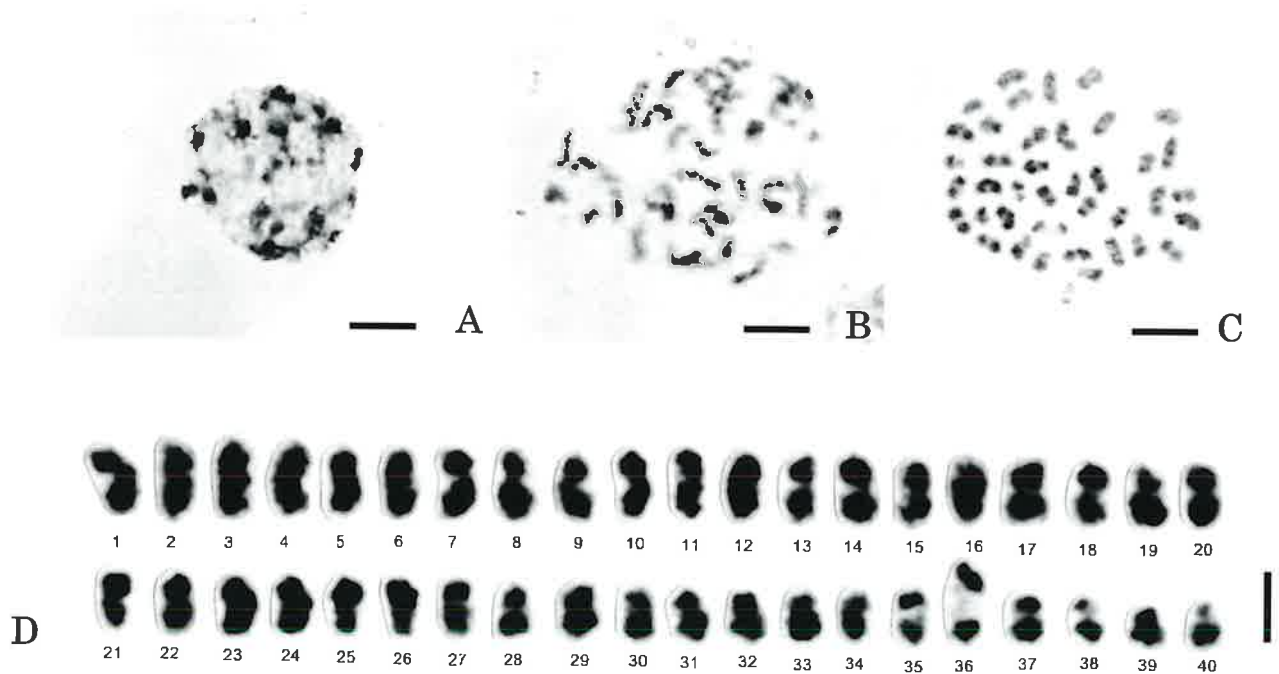


Fig. 2. Chromosomes of *Acineta superba*, HBG638,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

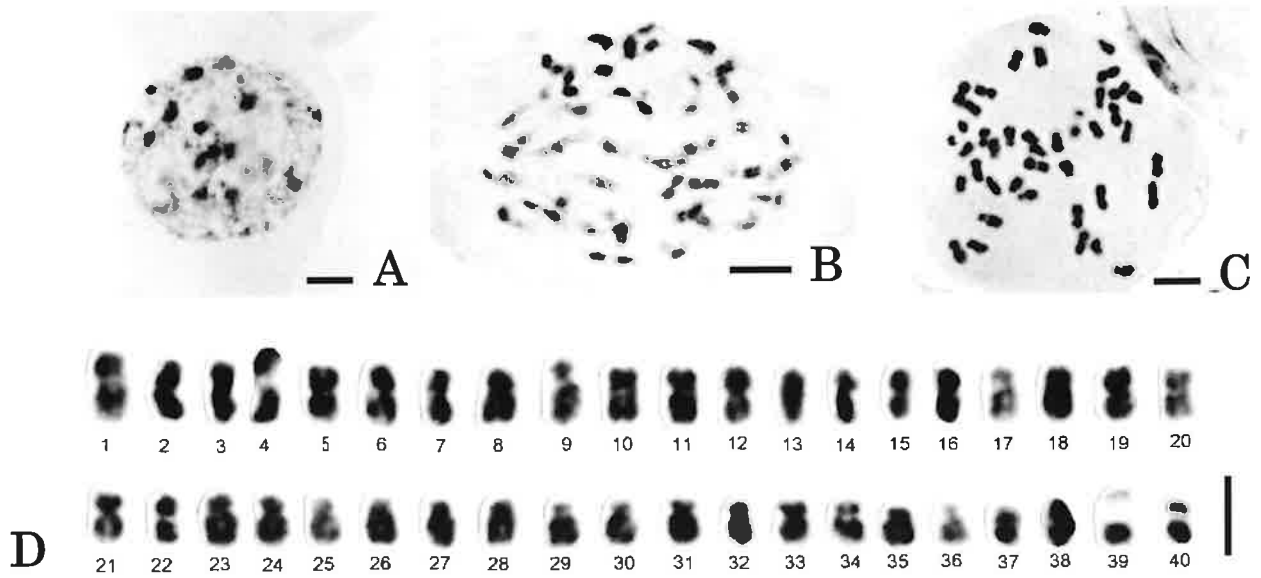


Fig. 3. Chromosomes of *Cirrhaea loddigesii*, HBG607,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

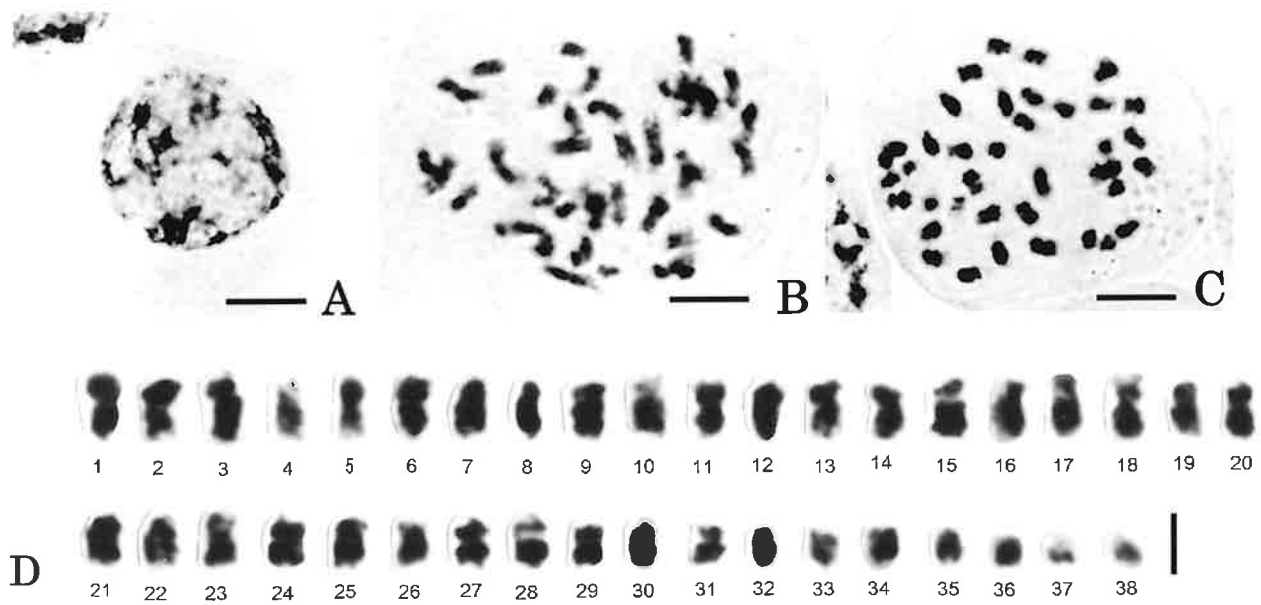


Fig. 4. Chromosomes of *Coeliopsis hyacinthosma*, HBG647,  $2n=38$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.



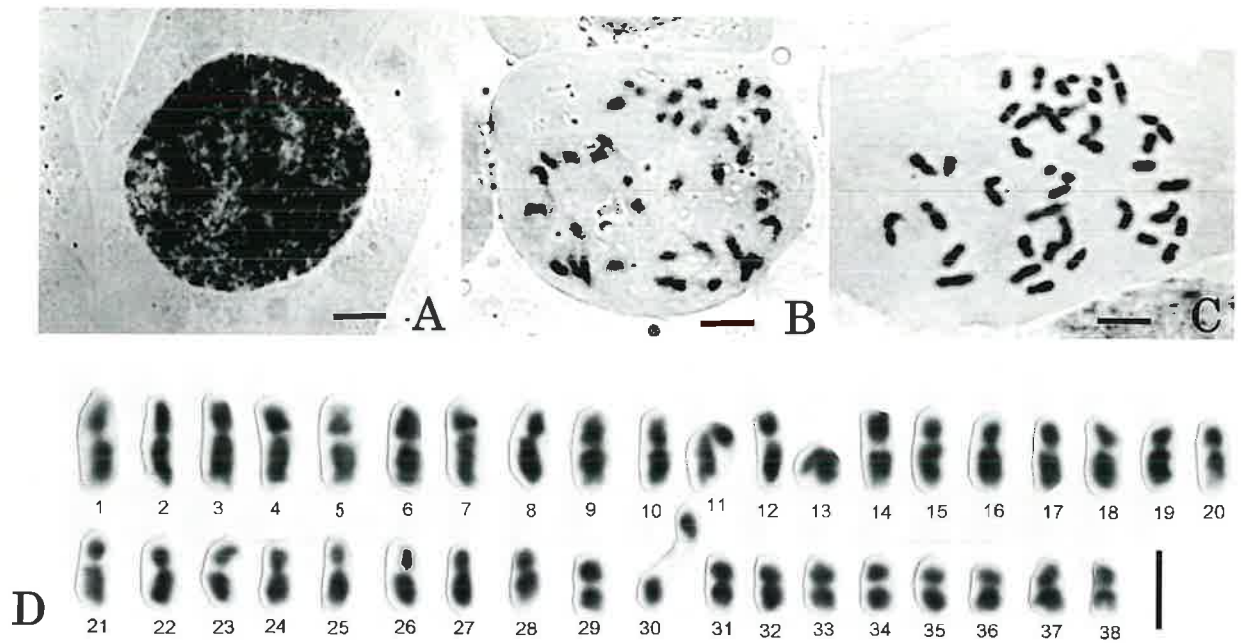


Fig. 5. Chromosomes of *Gongora armeniaca*, HBG563,  $2n=38$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

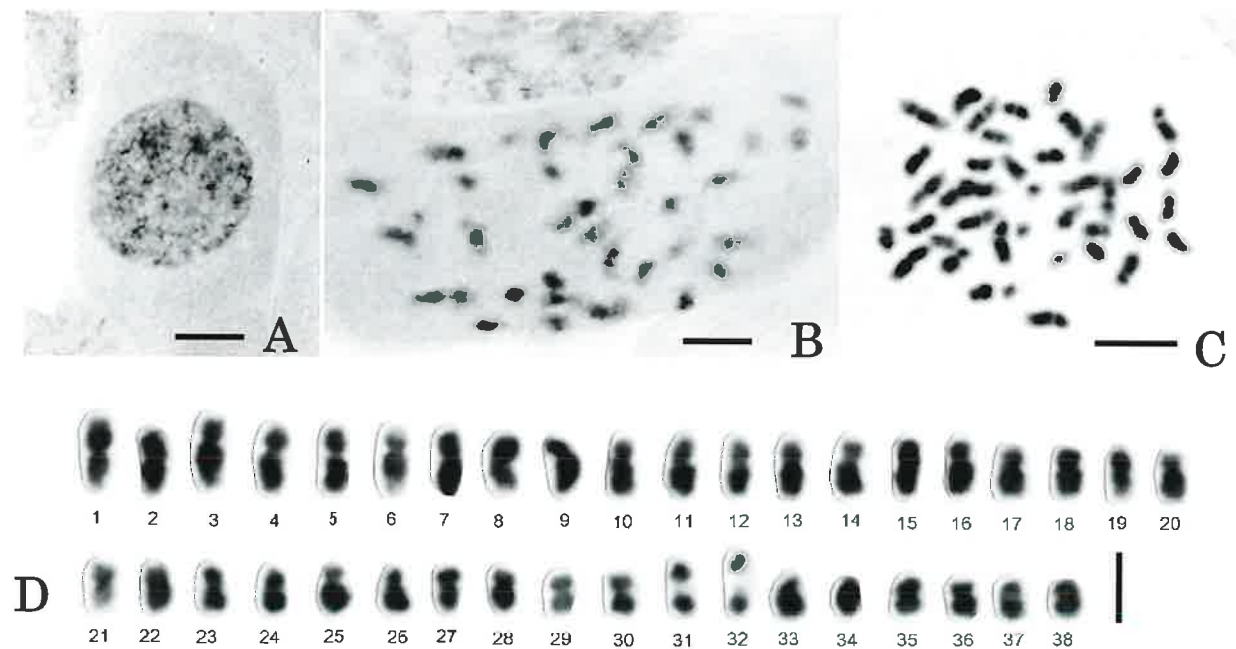


Fig. 6. Chromosomes of *Gongora truncata*, HBG602,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

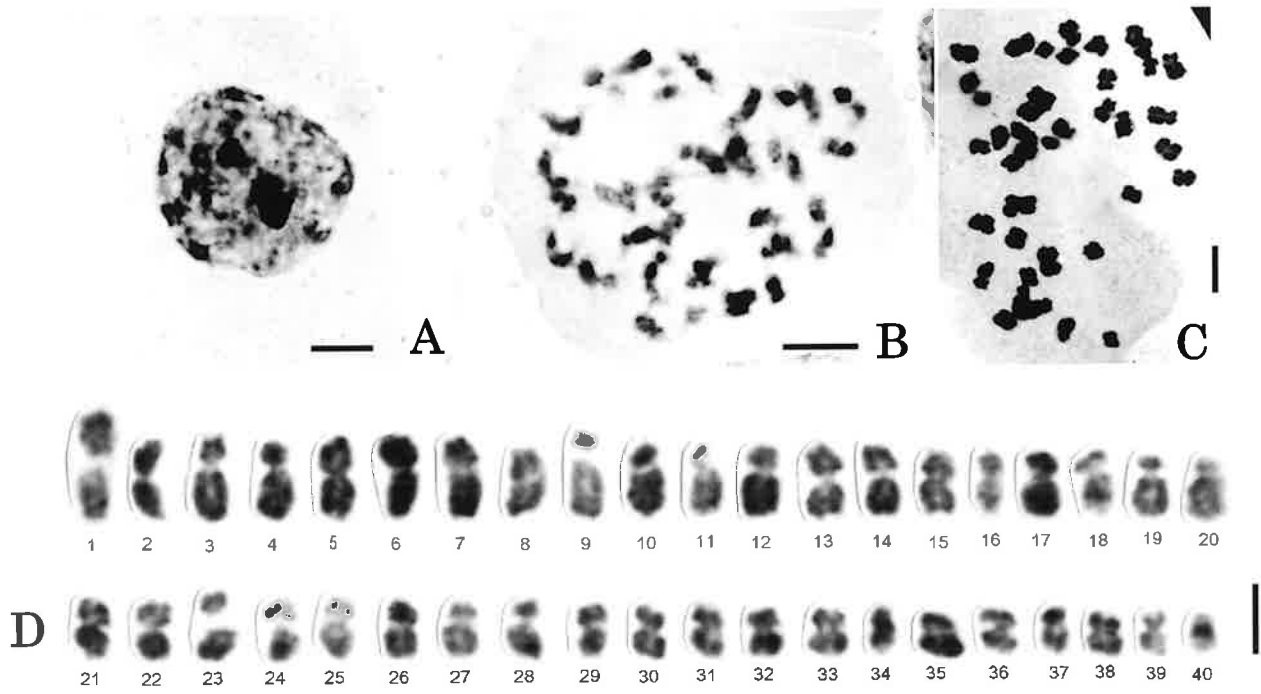


Fig. 7. Chromosomes of *Kegeliella atropilosa*, HBG2699,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

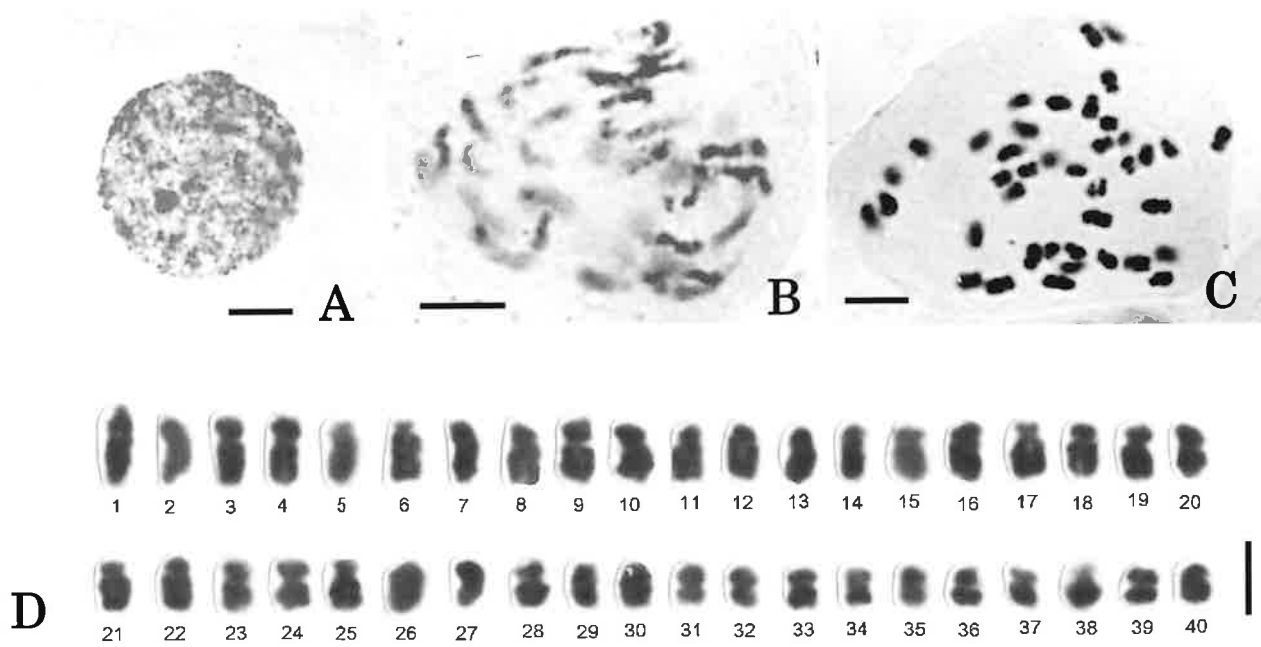


Fig. 8. Chromosomes of *Paphinia grandiflora*, HBG2220,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

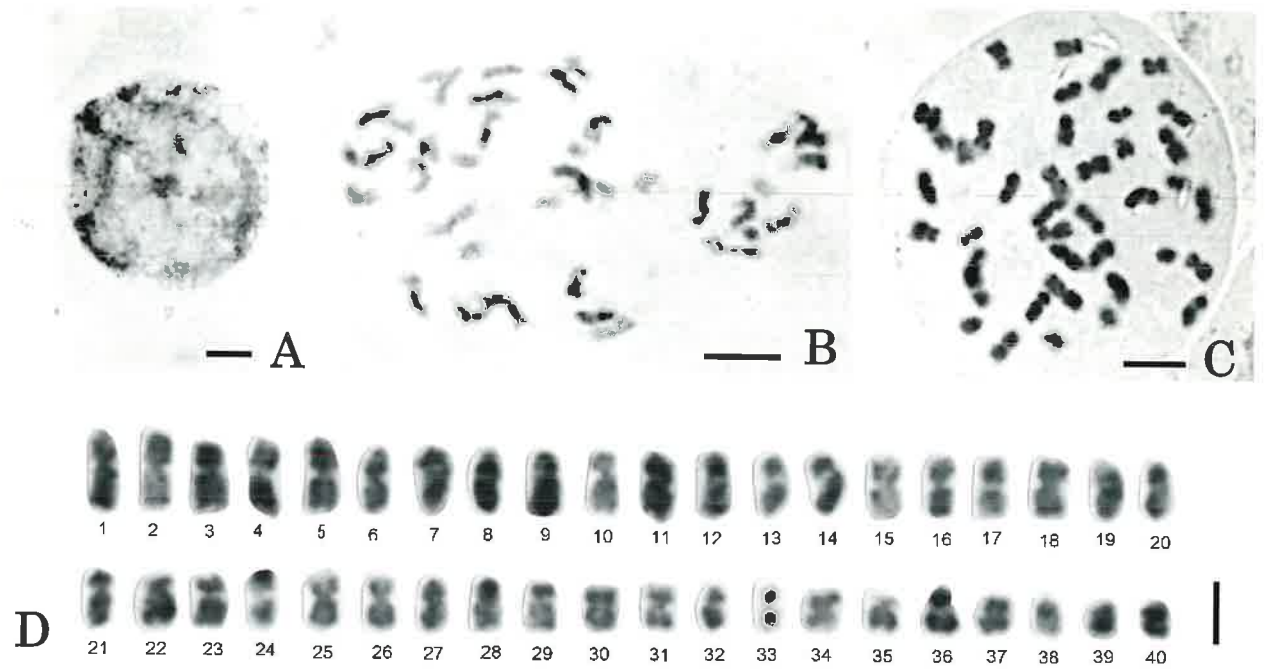


Fig. 9. Chromosomes of *Peristeria elata*, HBG621,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

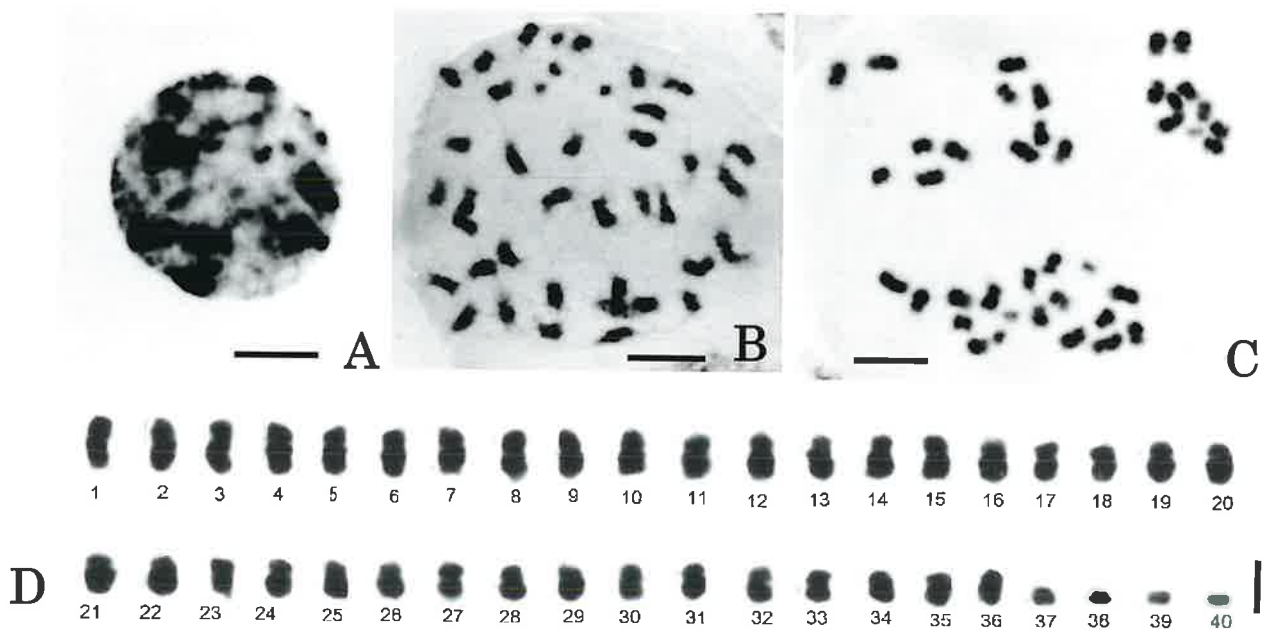


Fig. 10. Chromosomes of *Polycynis barbata*, HBG730,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

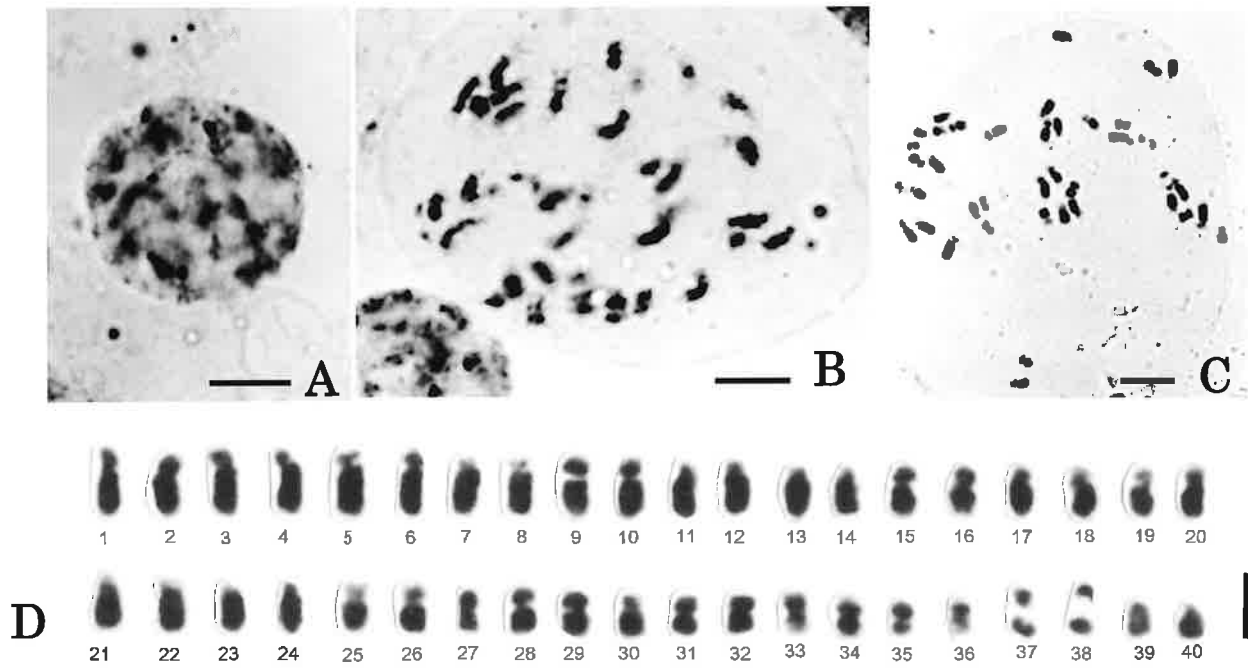


Fig. 11. Chromosomes of *Shlimia trifida*, HBG2819,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

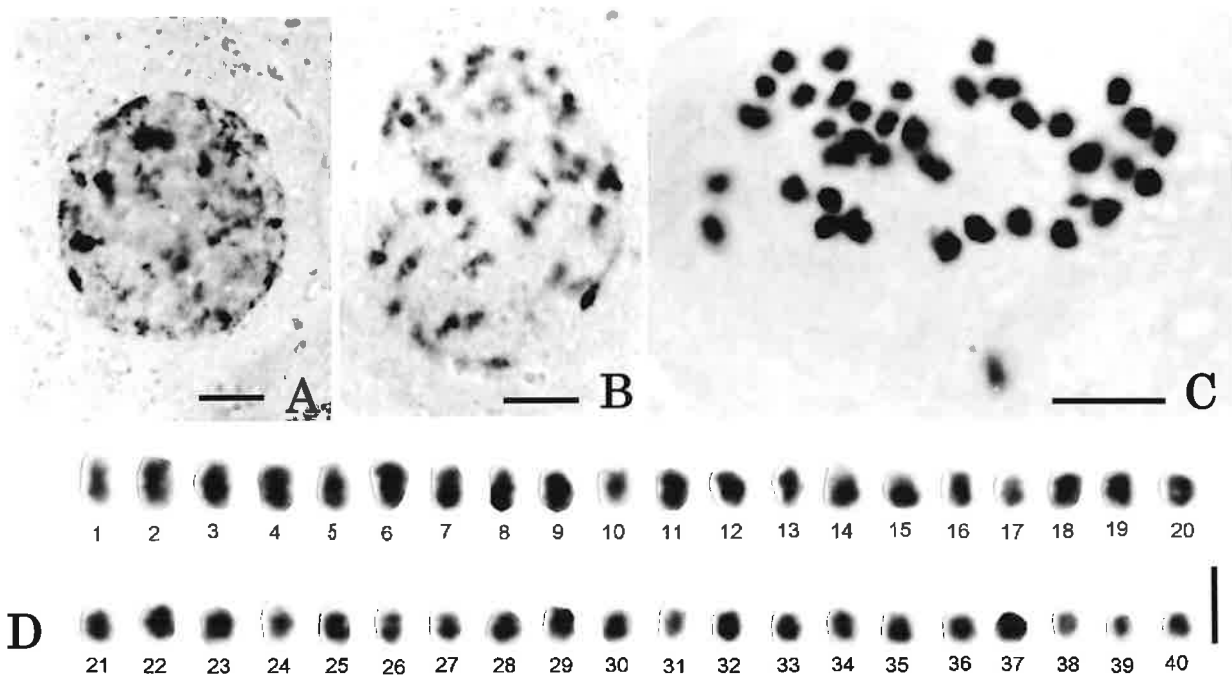


Fig. 12. Chromosomes of *Stanhopea cirrhata*, HBG2272,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

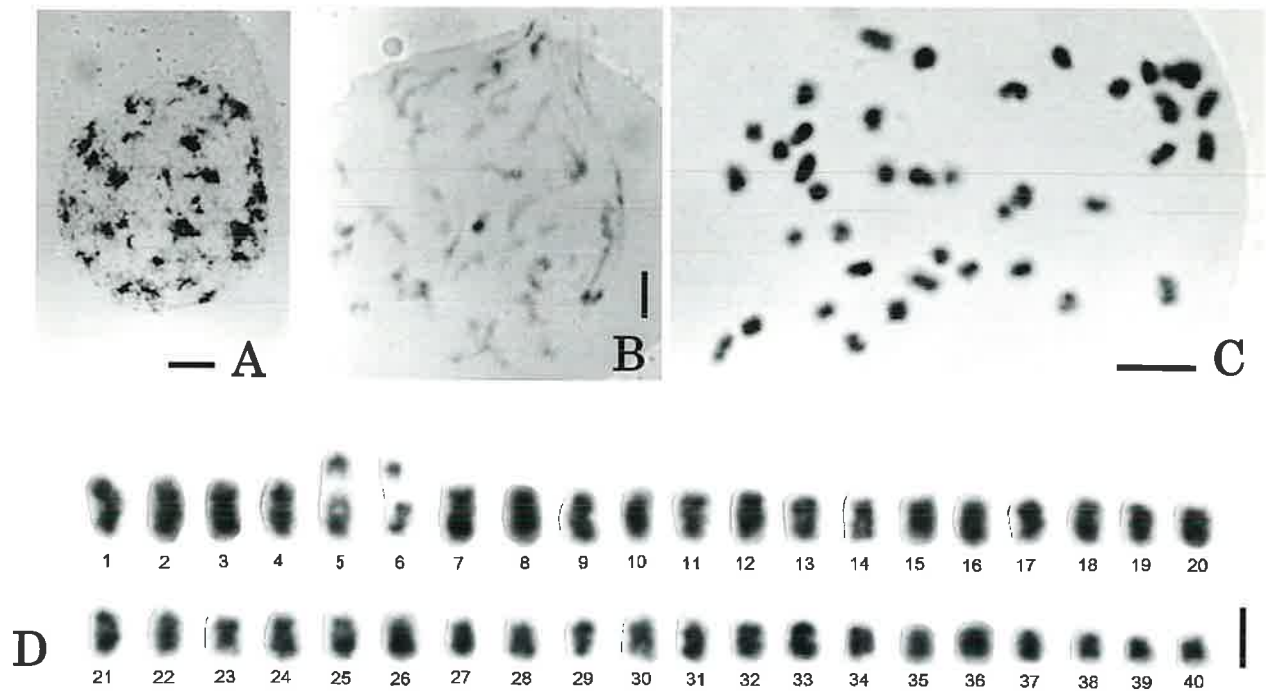


Fig. 13. Chromosomes of *Stanhopea guttulata*, HBG604,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

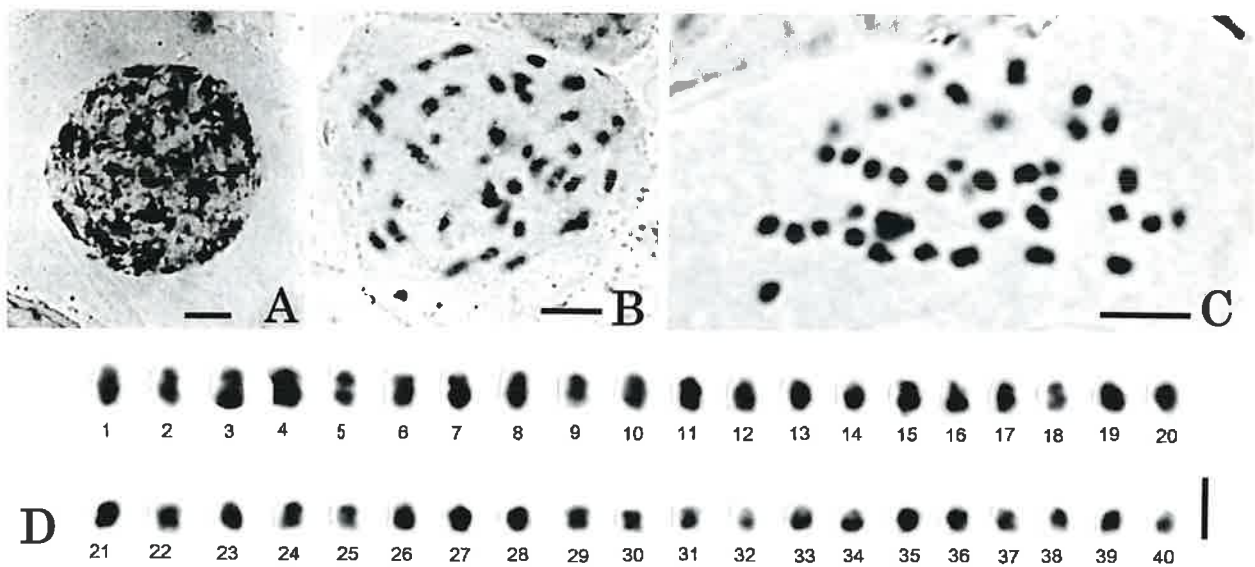


Fig. 14. Chromosomes of *Stanhopea pulla*, HBG668,  $2n=40$ . A, resting stage. B, mitotic prophase. C and D, mitotic metaphase. Bars indicate  $5\mu\text{m}$  in A-C and  $2\mu\text{m}$  in D.

名 称	広島市植物公園紀要第 30 号
主 管 課 所 在 地	財団法人広島市動植物園・公園協会植物公園 広島市佐伯区倉重三丁目 495 〒 731-5156 TEL(082)922-3600
発行年月日	平成 24 年 3 月 31 日
印刷会社名	株式会社 ニシキプリント



広島市植物公園 紀要

第30号

2012

広島市植物公園