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RESEARCH PAPER

Comparative Cytogenetics of Two *Squalius* Bonaparte, 1837 Species (Cypriniformes: Leuciscidae)

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Abstract
The karyotypes and other chromosomal characteristics of Anatolian endemic species *Squalius carinus* Özuluğ and Freyhof (Ichthyol Explor Freshw 22(2):107–148, 2011) and *S. fellowesii* (Günther, 1868) (Cypriniformes: Leuciscidae) were analysed by means of sequential Giemsa staining, C-banding and silver staining. The diploid chromosome numbers were invariably $2n = 50$; their karyotypes were composed of 12 pairs of metacentric, ten pairs of submetacentric and three pairs of subtelo-acrocentric chromosomes in *S. carinus,* whereas ten pairs of metacentric, ten pairs of submetacentric and five pairs of subtelo-acrocentric chromosomes in *S. fellowesii*. No heteromorphic sex chromosomes were detected. The largest chromosome pair in their karyotypes was a subtelo-acrocentric chromosome. C-positive heterochromatins were observed on the pericentromeric regions of most of the chromosomes in the studied species. In addition, heterochromatic blocs were observed in the karyotype of *S. carinus*. The nucleolus organizer regions were detected terminally on the short (p) arms of single submetacentric chromosome pair in both species. Our study thus confirmed overall conservatism of leuciscin karyotypes.

Keywords Fish cytotaxonomy · Karyotype stasis · NOR phenotype · Chromosome banding · Anatolian ichthyofauna

1 Introduction

Family Leuciscidae is one of the most widely distributed and highly diverse groups of cyprinoids (Schönhuth et al. 2018; Tan and Armbruster 2018). The genus *Squalius* belongs to this family and contains at least 21 species in the inland waters of Turkey where *Squalius carinus* and *S. fellowesii* are endemic species: the former one in Işıklı Lake, whereas the later in south-western Anatolia (Çiçek et al. 2015).

Only four species of the genus *Squalius* from Anatolia have been studied karyologically, namely *S. cephalus*, *S. orientalis*, *S. anatolicus* and *S. seyhanensis* (Pekol 1999; Kılıç-Demirok 2000; Ünal 2011; Unal and Gaffaroğlu 2016). There is no report about karyological data of *S. carinus* and *S. fellowesii*. Therefore, this study reports on the karyotype and chromosomal characteristics of Anatolian leuciscins *S. carinus* and *S. fellowesii* by means of sequential Giemsa staining, C-banding and silver staining.

2 Materials and Methods

2.1 Fish Sampling and Chromosome Preparation

Ten individuals of *S. carinus* were collected from Spring Işıklı, Denizli, Turkey (38°19′ N, 29°51′ E), four individuals of *S. fellowesii* were collected from stream at Karasandıklı, Afyon, Turkey (38°31′ N, 30°30′ E), five individuals were collected from Küfi Creek, Denizli, Turkey (38°21′ N, 29°50′ E), and six individuals were collected from Tabakdere, Salihli, Manisa, Turkey (38°28′ N, 28°03′ E). The individuals were carried alive to the laboratory and kept in the well-aerated aquarium until analysis. Species identification followed Özuluğ and Freyhof (2011). The chromosomal study was carried out after permission from the Kırşehir Ahi Evran University Local Ethics Committee for Animal Experiments (permit number:

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68429034/19). The air-drying technique of Bertollo et al. (2015) was performed from the head kidney for chromosome preparations. At least ten slides were prepared from each individual. Some slides were stained by 5% Giemsa. After analysis, the individuals were deposited as vouchers in 70% ethanol at the Cytogenetics Laboratory of the Faculty of Arts and Sciences of the Kırşehir Ahi Evran University, Kırşehir, Turkey, under the collection numbers MKA 96–120.

2.2 Chromosome Bandings: C-banding and Silver Staining

The C-banding technique of Sumner (1972) was used for visualization of constitutive heterochromatin regions, whereas the silver staining technique of Howell and Black (1980) was followed for determining NORs.

2.3 Microscopy, Image Processing and Karyotyping

The chromosomes were scanned with a Leica DM 3000 microscope (Leica Microsystems GmbH, Germany), and photographs of metaphases were taken with AKAS software (Argenit Mikrosistem, Turkey). Chromosomes were measured with digital calliper, and karyotype was arranged manually. Chromosomes were classified according to Levan et al. (1964). For calculating the NF (fundamental number), meta- and submetacentric chromosomes were taken as biarmed, whereas subtelo-acrocentric chromosomes were taken as uniarmed. Image processing was performed in Adobe Photoshop CS6.

3 Results

The diploid chromosome numbers of *S. carinus* and *S. fellowesii* were invariably $2n = 50$ (Figs. 1a and 2a). Karyotypes were composed as follows: 12 pairs of metacentric (m), ten pairs of submetacentric (sm) and three pairs of subtelo-acrocentric (st-a) chromosomes in *S. carinus* (Fig. 1b); ten pairs of m, ten pairs of sm and five pairs of st-a chromosomes in *S. fellowesii* (Fig. 2b). The NF was 94 in *S. carinus* and 90 in *S. fellowesii*. The largest chromosome pair of the complements was a st-a chromosome in the two species. Heteromorphic sex chromosomes were not detected in karyotype of *S. carinus* and nor that of *S. fellowesii*. Blocks of C-positive heterochromatins were found on the pericentromeric regions of the most of the chromosomes (Figs. 1c, d and 2c, d). Also, heterochromatic blocs were observed in No. 23 and No. 25 in the karyotype of *S. carinus* (Fig. 1d). Positive Ag–NOR sites were observed terminally on the p arms of one sm chromosome pair (Figs. 1e and 2e), located on pair No. 16 in *S. carinus* (Fig. 1f) and No. 12 in *S. fellowesii* (Fig. 2f).

4 Discussion

Four from 21 Anatolian species of the genus *Squalius* so far karyologically studied indicated a invariable $2n = 50$. Their karyotypes were dominated by biarmed chromosomes (Table 1). The two species examined herein also had $2n = 50$ chromosomes and karyotypes also dominated by biarmed chromosomes. However, their karyotypes showed some differences compared to the previous reports. The karyotype of *S. carinus* differed from that of *S. fellowesii* by two more biarmed chromosome pairs. The number of biarmed chromosomes of *S. carinus* was the same as in *S. seyhanensis* (Unal and Gaffaroğlu 2016). However, the number of biarmed chromosomes in karyotypes of *S. carinus* and *S. fellowesii* differed from that of *S. cephalus* (Pekol 1999), *S. orientalis* (Kılıç-Demirok 2000) and *S. anatolicus* (Ünal 2011) by having more biarmed chromosomes. According to these reported differences, the NF values of *S. carinus* and *S. fellowesii* were higher compared to *S. cephalus* (Pekol 1999), *S. orientalis* (Kılıç-Demirok 2000) and *S. anatolicus* (Ünal 2011). Otherwise, the NF value of *S. fellowesii* was lower compared to that of *S. carinus* and *S. seyhanensis* (Unal and Gaffaroğlu 2016). Different NF values of these species and/or the same NF but different chromosome morphologies may be the result of pericentromeric inversions (and/or translocations involving centromeres) as reported by Ayata et al. (2016) in karyotypes of Anatolian *Pseudophoxinus* species.

The largest chromosome pair (st-a) which has been determined as leuciscine cytotaxonomic marker (Rab et al. 2008) was detected in this study too. Thus, the karyotypes of *S. carinus* and *S. fellowesii* were also similar to those of other Anatolian *Squalius* species (Ünal 2011; Unal and Gaffaroğlu 2016). Moreover, karyological patterns ($2n$, the marker chromosome and dominance of m and sm chromosomes with a reduced number of uniarmed chromosomes) found in this study were the same as the reports in other European *Squalius* species—*S. carolitertii*, *S. pyrenaicus* (Collares-Pereira et al. 1998), *S. lucumonis* (Rossi et al. 2012), *S. aradensis* and *S. torgalensis* (Nabais et al. 2013).

Heteromorphic sex chromosomes have not been detected in karyotypes of *S. carinus* and *S. fellowesii* as not observed in that of other Anatolian and European *Squalius* species (Pekol 1999; Kılıç-Demirok 2000; Ünal 2011; Rossi et al. 2012; Nabais et al. 2013; Unal and Gaffaroğlu 2016).

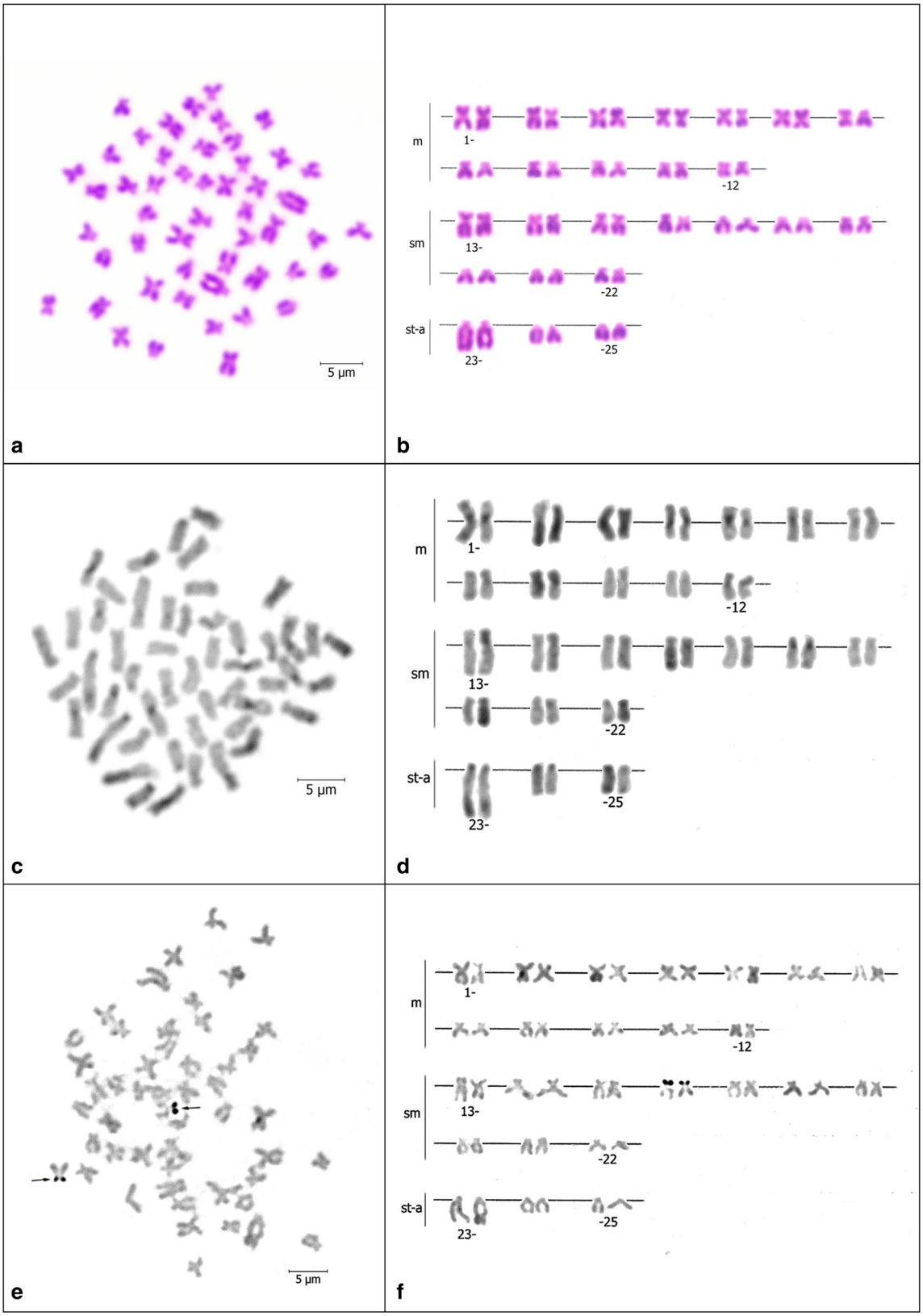

Fig. 1 Giemsa stained metaphase (**a**), the corresponding karyotype (**b**), C-banded metaphase (**c**), the corresponding karyotype (**d**), silver-stained metaphase (**e**) and the corresponding karyotype (**f**) of *Squalius carinus*. Arrows indicate the Ag–NORs. Scale bar = 5 µm

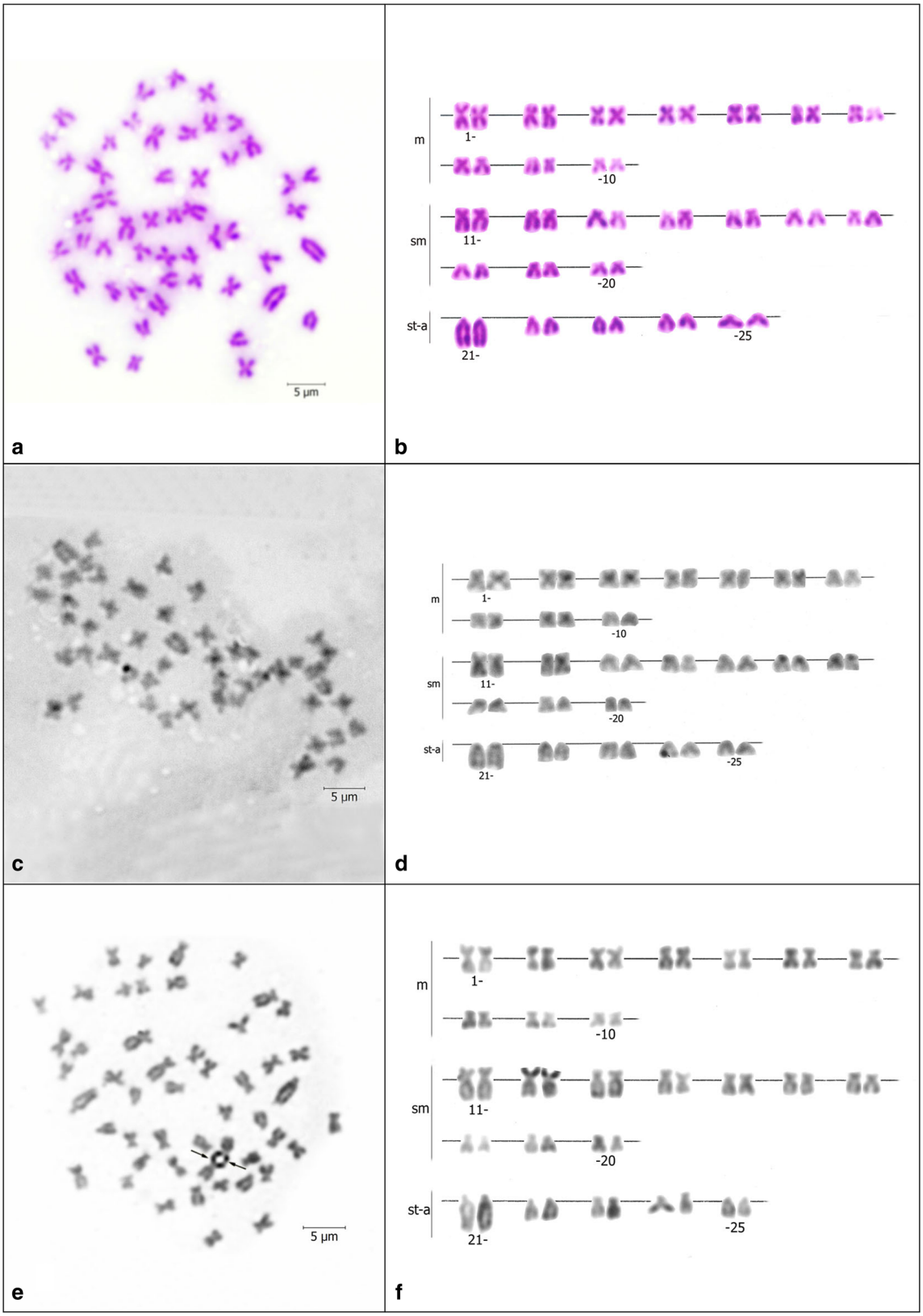

Fig. 2 Giemsa stained metaphase (**a**), the corresponding karyotype (**b**), C-banded metaphase (**c**), the corresponding karyotype (**d**), silver-stained metaphase (**e**) and the corresponding karyotype (**f**) of *Squalius fellowesii*. Arrows indicate the Ag–NORs. Scale bar = 5 µm

Table 1 Cytogenetic data for the representatives of the genus *Squalius* from Anatolia, Turkey

Species	2*n*	Chromosome morphology	NF	References
S. cephalus	50	18m + 12sm + 20st-a	80	Pekol (1999)
		20m + 12sm + 18st-a	82	
S. orientalis	50	14m + 20sm + 16st-a	84	Kılıç-Demirok (2000)
S. anatolicus	50	10m + 22sm + 10st + 8a	82	Ünal (2011)
S. seyhanensis	50	16m + 28sm + 6st-a	94	Unal and Gaffaroğlu (2016)
S. carinus	50	24m + 20sm + 6st-a	94	This study
S. fellowesii	50	20m + 20sm + 10st-a	90	This study

2n diploid chromosome number; *NF* fundamental number; *m* metacentric; *sm* submetacentric; *st-a* subtelo-acrocentric

The C-banding technique reveals the blocks of constitutive heterochromatin. These regions include highly and moderately repetitive DNA. These bands usually were located on the centromere of chromosomes and pericentromeric, telomeric and sometimes intercalary regions. C-bands can be heteromorphic in many species and also among individuals and populations of the same species (Salvadori et al. 2015). C-banding technique was applied to only *S. anatolicus* (Ünal 2011) and *S. seyhanensis* (Unal and Gaffaroğlu 2016) from Anatolia to date. The karyotypes of *S. carinus* and *S. fellowesii* showed mainly pericentromeric C-bands as reported in other Anatolian *Squalius* species (Ünal 2011; Unal and Gaffaroğlu 2016). The present study also showed similar patterns, in accordance with *S. carolitertii*, *S. pyrenaicus* (Collares-Pereira et al. 1998) and *S. lucumonis* (Rossi et al. 2012). Heterochromatic blocs were observed only in *S. carinus* but not in *S. fellowesii*. The heterochromatic bloc located on the marker st-a chromosome in *S. carinus* was terminal, as in *Vimba* (Rábová et al. 2003), *Rutilus*, *Scardinius* (Bianco et al. 2004), *Pseudochondrostoma*, *Achondrostoma* species (Pereira et al. 2009) and *S. lucumonis* (Rossi et al. 2012). Rab et al. (2008) and Pereira et al. (2009) reported that this result suggests its AT-rich repetitive DNA character. Additionally, the other heterochromatic bloc may be a putative *S. carinus* species-specific marker.

Ag–NORs are useful as cytogenetic markers (Zaleśna et al. 2017). The Ag–NOR number and locations were proven to be species specific, although inter-individual Ag–NOR variability has also been observed within species (Zaleśna et al. 2017). One Ag–NOR-bearing chromosome pair (located on the terminal regions of the p arms of sm chromosomes) observed in the karyotypes of *S. carinus* and *S. fellowesii* is the most common NOR phenotype among leuciscins (Collares-Pereira et al. 1998; Boron et al. 2009; Pereira et al. 2009). Within the genus *Squalius* from Anatolia, a single Ag–NOR-bearing chromosome pair was reported in *S. anatolicus* (Ünal 2011), *S. cephalus* (Pekol and Arslan 2014) and *S. seyhanensis* (Unal and Gaffaroğlu 2016) and in this study. However, the locations of Ag–NOR in st-a chromosome in *S. cephalus* (Pekol and Arslan 2014) were different from *S. carinus* and *S. fellowesii*. Furthermore, the number and location of the Ag–NOR of *S. carinus* and *S. fellowesii* were similar to European leuciscins like *S. carolitertii*, *S. pyrenaicus* (Collares-Pereira et al. 1998), *S. lucumonis* (Rossi et al. 2012), *S. aradensis* and *S. torgalensis* (Nabais et al. 2013).

In conclusion, cytogenetic data for *S. carinus* and *S. fellowesii* were presented here for the first time. The results of the present study and those described in the literature showed that $2n = 50$ chromosomes were conservative in this genus but their karyotype variability is higher. Studies on different species of this genus should be useful to a better understanding of the karyotypic differentiation of its Anatolian representatives. This study provides important knowledge of Anatolian leuciscin chromosomes and may be useful in their cytotaxonomy.

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