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Karyomorphology of Three *Halophila* species (Hydrocharitaceae, Alismatales) from Haad Chao Mai National Park, Trang Province, Thailand

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ABSTRACT

The taxonomic identification of some species of *Halophila*, a marine angiosperm genus in the Hydrocharitaceae family, remains complicated by morphological plasticity. Therefore, the karyotypes were determined for three *Halophila* species from Haad Chao Mai National Park, Thailand: *H. beccarii*, *H. major* and *H. ovalis*. The results showed that the chromosome numbers of all the studied *Halophila* were $2n = 18$. However, there were inter-specific differences in the karyotype formulae. The karyotype formulae of *H. beccarii*, *H. major* and *H. ovalis* ($2n = 6m + 4sm + 8st$, $2n = 8m + 6sm + 4st$, and $2n = 8m + 4sm + 6st$, respectively) were distinctly different from those found in previous studies of specimens from Hong Kong, China and India. This indicates biogeographically karyotypic variation among populations. This is the first report of karyotypes of three *Halophila* species from Thailand. Several karyotype asymmetry indices were also examined. According to karyotype asymmetry index (A), *Halophila beccari* had the most asymmetrical karyotype ($A = 0.33$) that agreed with the highest subtelocentric chromosomes. These karyotype features can be applied to further studies, for example, taxonomy, physiology and chromosome evolution.

Keywords: chromosome, *Halophila*, Haad Chao Mai National Park, karyotype

1. INTRODUCTION

Halophila, a genus of seagrass, belongs to family Hydrocharitaceae. Species in this genus have been distributed worldwide and play the important role in the marine ecological system. Five *Halophila* species, *H. beccari*, *H. decipiens*, *H. major*, *H. minor* and *H. ovalis*, have been reported from Thailand [23]. The largest seagrass beds in Thailand are located in the northern part of the Haad Chao Mai National Park and cover 18 km^2. *Halophila* is a common and dominant species in this area [15]. However, *Halophila* is well-known for its vast morphological variations (e.g. leaf size and shape) and it is difficult to identify species using only their morphology.

Chromosome number and karyotype formula are characters that have been widely used in plant systematics and the study of evolution [6, 9, 20, 27]. Chromosome number is a good taxonomic character for the classification of organisms into taxa, and the morphology of the chromosome can also indicate differences or similarities between taxa [4]. It has been shown that seagrasses from different genera or even families can have the same chromosome number. For example, the Hydrocharitaceae: *Halophila stipulacea* (Forssk.) Asch., *Halophila beccarii* Asch., *Halophila ovalis* (R.Br.) Hook.f.*, Thalassia hemprichii* (Ehrenb. ex Solms) Asch. and *Enhalus acoroides* (L.f.) Royle shared the same chromosome number, $2n = 18$, whereas the chromosome number of other species of Hydrocharitaceae, *Cymodocea nodosa* (Ucria) Asch., *Cymodocea serrulata* (R.Br.) Asch. & Magnus (Cymodoceaceae), and *Halophila minor* (Zoll.) Hartog, was $2n = 28$ [4, 5, 7, 11, 22, 24, 29]. Regarding the karyotype formulae of different genera, although similarities have been identified between, for example, *E. acoroides* and *T. hemprichii* ($2n = 18$ ($12m + 6sm$)) [28]. The karyotype formula of each *Halophila* species displayed a unique pattern: $2n = 18$ ($8m + 10sm$) for *H. beccarii*, $2n = 18$ ($4m + 14\ sm$) for *H. ovalis*, and $2n = 28$ ($16m + 8sm + 4st$) for *H. minor* [28, 29]. The aim of this study was to investigate the chromosome number and karyotype formulae of three *Halophila* species, *H. beccarii*, *H. major* (Zoll.) Miq., and *H. ovalis*, populations from Haad Chao Mai National Park, Thailand for aid species level identification. Moreover, biogeographical variation can be found among seagrass populations in order to obtain fundamental data for further studies (e.g. taxonomy, physiology and evolution).

2. MATERIALS AND METHODS

Shoot tips of *Halophila beccarii*, *H. major* and *H. ovalis* were collected from one large population of Haad Chao Mai National Park, Trang Province, Thailand (Latitude: 7.3846º, Longitude: 99.3336º). At least ten cells per individual and three plants per species were examined. Preliminary studies showed that the period between 11 a.m. and 12 a.m. was the most suitable time for sampling, as metaphase can often be observed. Shoot tips were pretreated in saturated *para*-dichlorobenzene (PDB) solution for 5 to 6 hours at 12 ºC. Afterwards, they were fixed in Carnoy's solution (3:1 mixture of 95% ethanol and glacial acetic acid) for 24 hours. The shoot tips were washed 3 times in 95% ethanol and subsequently subjected to the Feulgen squash technique by hydrolysis in 1 N HCl for 3 to 5 minutes at 60 ºC. Samples were then stained with Carbol Fuchsin for 5 to 6 minutes at room temperature to determine chromosome number. A total of ten of the best metaphase cells were counted, measured, and photographed under a light microscope (Olympus BX51).

Chromosome types were classified using the arm ratio (AR) according to Levan et al [13]. Chromosomes with arm ratios ranging from 1.0 to 1.7 were defined as metacentric (*m*) while those with arm ratios from more than 1.7 to 3.0 were classified as submetacentric (*sm*). Subtelocentric chromosomes (*st*) were defined by arm ratios ranging from more than 3.0 to 7.0 and telocentric chromosomes (*t*) by arm ratios is more than 7.0. The relative length of chromosome (RL%) was expressed as a percentage calculated using the following formula: RL% = chromosome length × 100/total chromosome lengths. The centromeric index (CI) was calculated by ratio of short arm length and total arm length. The standard deviation (SD) of arm ratio, relative length and centromeric index were also computed.

Other karyotype parameters reflected to the karyotype asymmetry were calculated i.e. intrachromosomal asymmetry index (A1), interchromosomal asymmetry index (A2) by Romero Zarco [19] , karyotype asymmetry index (A) by Watanabe et al. [25], coefficient of variation of chromosome length (CV_{CL}), coefficient of variation of the centromeric index (CV_{CI}) by Paszko [17] and mean centromeric asymmetry (M_{CA}) by Peruzzi and Eroğlu [18].

3. RESULTS AND DISCUSSION

Karyomorphology of Thai *Halophila, H. beccarii, H. major*, and *H. ovalis,* were examined from the large population of Haad Chao Mai National Park, Trang Province, Thailand. The relative length (RL%), arm ratio (AR) and chromosome type in each chromosome pair of all studied species were shown in Table 1. Chromosome number, karyotype formulae and other karyotype parameters were presented in Table 2.

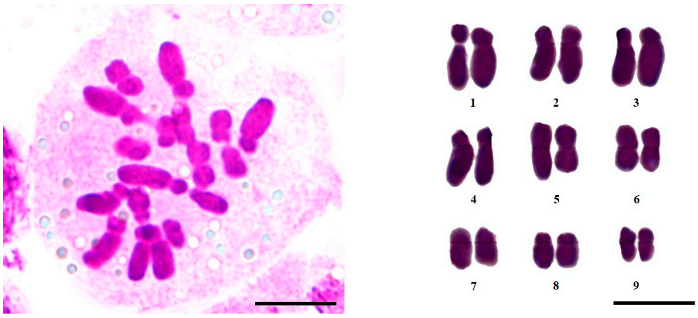
Table 1. Average relative length (RL%), arm ratio (AR), standard deviation (SD) and chromosome type (chro. Type) in *H. beccarii* Asch., *H. major* (Zoll.) Miq. and *H. ovalis* (R.Br.) Hook.f. (n=10).

Chro. pair	***H. beccarii* Asch.**			***H. major* (Zoll.) Miq.**			***H. ovalis* (R.Br.) Hook.f.**		
	RL%±SD	**AR±SD**	**Chro. type**	**RL%±SD**	**AR±SD**	**Chro. type**	**RL%±SD**	**AR±SD**	**Chro. type**
1	8.63±0.73	3.39±0.46	st	9.43±0.88	1.96±0.22	sm	9.07±1.02	2.05±0.48	sm
2	7.67±0.91	3.07±0.44	st	7.03±0.62	3.70±0.19	st	7.58±0.41	3.21±0.78	st
3	6.82±0.16	3.11±0.68	st	6.48±0.55	3.38±0.55	st	6.64±0.25	3.57±0.46	st
4	5.60±0.38	3.49±0.82	st	5.85±0.69	2.23±0.61	sm	5.52±0.44	3.15±0.78	st
5	4.99±0.45	1.21±0.14	m	5.24±0.36	1.23±0.20	m	5.41±0.24	1.28±0.14	m
6	4.45±0.36	1.20±0.06	m	4.68±0.34	1.20±0.16	m	4.57±0.32	1.16±0.22	m
7	4.14±0.34	1.77±0.37	sm	4.16±0.40	1.81±0.33	sm	3.94±0.37	2.08±0.31	sm
8	3.79±0.34	1.97±0.10	sm	3.88±0.37	1.28±0.14	m	3.90±0.19	1.16±0.11	m
9	3.90±0.19	1.23±0.17	m	3.26±0.22	1.23±0.07	m	3.37±0.25	1.17±0.10	m

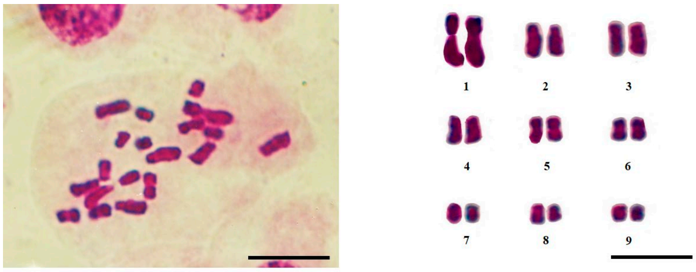
Table 2. Karyotype parameters from *H. beccarii* Asch., *H. major* (Zoll.) Miq. and *H. ovalis* (R.Br.) Hook.f.

Karyotype parameters	***H. beccarii* Asch.**	***H. major* (Zoll.) Miq.**	***H. ovalis* (R.Br.) Hook.f.**
Chromosome number (2n)	18	18	18
ploidy	2x	2x	2x
Karyotype formula (KF)	6*m* + 4*sm* + 8*st*	8*m* + 6*sm* + 4*st*	8*m* + 4*sm* + 6*st*
Arm ratio (AR)	1.199 - 3.485	1.200 - 3.705	1.115 - 3.567
Relative length (RL%)	3.794 - 8.631	3.263 - 9.431	3.372 - 9.068
Centromeric index (CI)	0.231 - 0.460	0.213 - 0.458	0.222 - 0.466
Intrachromosomal asymmetry index (A1)	0.54	0.47	0.48
Interchromosomal asymmetry index (A2)	0.07	0.16	0.12
Karyotype asymmetry index (A)	0.33	0.27	0.28
Coefficient of variation of chromosome length (CV_{CL})	6.92	16.35	12.00
Mean centromeric asymmetry (M_{CA})	32.52	27.00	28.00
Coefficient of variation of the centromeric index (CV_{CI})	30.24	27.26	29.96

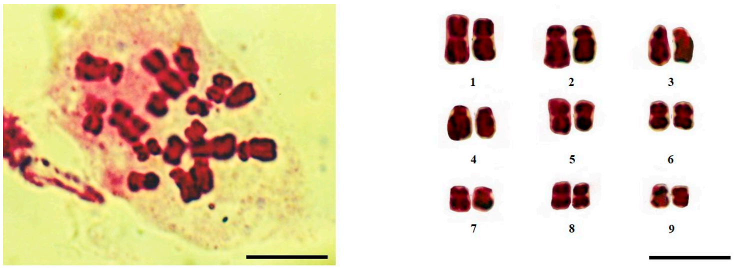
This study revealed the consistent chromosome numbers in shoot tips of these *Halophila* species. All species shared the same somatic chromosome number of $2n = 18$ (Table 1, 2 and Figure 1) and agreed with the previous reports [4, 7, 24, 29]. A few researches showed different chromosome numbers in some *Halophila* species, for instance, *H. minor* and *H. ovata* (synonym of *H. minor*) were $2n = 28$ and $2n = 16$, respectively [22, 28]. According to earlier studies [2, 3, 10, 12], the basic chromosome number in seagrass was proposed to $x = 9$, all three species of *Halophila* in Thailand should be diploidy ($2n = 2x = 18$). From the result, chromosome number was not a useful parameter for species differentiation. A similar conclusion was reached by Babcock [1], who mentioned that



(A) *Halophila beccarii* Asch.



(B) *Halophiola major (*Zoll.) Miq.



(C) *Halophila ovalis* (R.Br.) Hook.f.

Figure 1. Metaphases $2n$ =18 and karyograms of *H. beccarii* Asch. (A), *H. major* (Zoll.) Miq. (B) and *H. ovalis* (R.Br.) Hook.f. (C); scale bar = 10 µm.

chromosome number in itself is an insufficient character to establish the relationship between groups within the plant kingdom.

In this investigation, the karyotypes of three *Halophila* species from Haad Chao Mai National Park, Trang Province were different (Figure 1). All three species displayed an asymmetrical karyotype including metacentric (*m*), submetacentric (*sm*), and subtelocentric (*st*) chromosomes. The karyotype features used for this work consisted of the average relative length (± SD), arm ratio (± SD) and type of each chromosome pair of *H. beccarii*, *H. major* and *H. ovalis* (shown in Table 1). Other karyotype parameter representing the karyotype asymmetry were presented as A1, A2, A, CV_{CI}, CV_{CL} and M_{CA} (Table 2).

The karyotype of *H. beccarii* consisted of three pairs of metacentric, two pairs of submetacentric and four pairs of subtelocentric chromosomes. The karyotype formula was $2n = 2x = 6m + 4sm + 8st$ (Table 1 and 2), which differs from the finding of a recent study [29]. Karyotype formulae for *H. beccarii* from Hong Kong, China and from India were shown as $2n = 2x = 8m + 10sm$ and $2n = 2x = 14m + 4sm$, respectively [24]. *Halophila beccarii* represented the arm ratio ranged between 1.199 and 3.485 and the centromeric index ranging from 0.231 to 0.460. The percentage of relative length was between 3.794 and 8.631.

The karyotype of *H. major* comprised four metacentric chromosome pairs, three submetacentric chromosome pairs, and two subtelocentric chromosome pairs. It is emphasized that the karyotype formula of *H. major*, $2n = 2x = 8m + 6sm + 4st$ (Table 1 and 2), is reported here for the first time. This result would be useful for comparative karyotype studies of *H. major*. The arm ratio ranged between 1.200 and 3.705 and the centromeric index ranging from 0.213 to 0.458. The percentage of relative length was ranged from 3.263 and 9.431.

For *Halophila ovalis*, the karyotype comprised four metacentric pairs, two submetacentric pairs, and three subtelocentric pairs. The karyotype formula was $2n = 2x = 8m + 4sm + 6st$ which differed from the previously reported formula ($2n = 2x = 4m + 14sm$) [19]. This species displayed the arm ratio ranged between 1.155 and 3.567 and the centromeric index ranging from 0.222 to 0.466. The percentage of relative length was between 3.372 and 9.068.

According to the percentage of relative length, arm ratio and chromosome type of each chromosome pair (Table 1), *H. major* is probably more closely related to *H. ovalis* than *H. beccarii*. Molecular phylogenetic studies derived from nuclear rDNA sequences revealed that *H. beccarii* was separated in different clade from the clade of *H. major* and *H. ovalis* [16, 26].

The differences between the karyotype formulae of *H. beccarii* and *H. ovalis* found in the present study and those reported from a recent study [29] may be due to differences in the habitats of the seagrasses. The karyotype can vary within a species depending on the presence of inversion and other chromosome structural changes [9, 21]. Beside on the chromosome number, the concept of karyotype asymmetry, with predominance of telocentric / subtelocentric chromosomes and highly heterogeneous chromosome size [14], is one of the essential feature to clarify the species delimitation and chromosomal change during evolution. From this studies, the number of telocentric chromosomes in *H. beccarii* was higher than those in *H. major* and *H. ovalis* presented the highest value of A1, A, M_{CA} and CV_{CI}. In contrast, *H. beccari* showed the lowest values of A2 and CV_{CL} reflected low variation in chromosome length in a complement.

Karyomorphological features of genus *Halophila* from various populations and localities in Thailand should be continued using chromosome banding, molecular cytogenetic techniques (FISH and GISH) complemented with conventional method for elucidate the species identification, genetic relationship and also karyotype evolution within this genus.

4. CONCLUSIONS

The chromosome numbers of three Thai *Halophila* species from Haad Chao Mai National Park, Trang Province, Thailand were the same, $2n = 18$. However, the karyotype formulae were different among species. The karyotype formulae of *H. beccarii*, *H. major* and *H. ovalis* were $2n = 6m + 4sm + 8st$, $2n = 8m + 6sm + 4st$, and $2n = 8m + 4sm + 6st$, respectively. *Halophila beccarii* presented higher karyotype asymmetry values and lower variation in chromosome length than *H. major* and *H. ovalis*. The differences in karyotype formulae from our results and recent studies indicated biogeographical karyotypic variation among populations.

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