

KARYOTYPE ANALYSIS ON SOME GREEK WILD SPECIES OF *HORDEUM* (*MARINUM* GROUP) AND *TAENIATHERUM*

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**Abstract:** The karyotypes of four diploid wild species of Gramineae (North-Greek in origin) were defined. Three of them belong to *Hordeum marinum* group, taken as a species in *sensu lato*, and they are namely: *H. marinum*, *H. marinum* I (morphological type) and *H. hystrix*. The fourth material is *Taeniatherum caput-medusae*. The karyotypes were determined qualitatively (chromosome morphology, centromere position, SAT pairs) and quantitatively (total chromosome length, arm lengths and variation of size). The analysis resulted in: 1) almost identical karyotypes between *H. marinum* and *H. marinum* I (similarity in chromosome morphology, absolute values and variation of size); 2) significant karyotypic similarity between *H. hystrix* and the two representatives of *H. marinum* (in arm ratios, SAT pairs and relative lengths) yet difference in quantitative features (higher values in *H. hystrix*); 3) different karyotypic synthesis of *T. caput-medusae* which was recognized as more symmetrical, with predominance of mesocentric chromosomes and different morphology of the SAT pair. This study corroborates the aspect that the three materials belong to the same species-aggregate (perhaps conspecific) whereas *Taeniatherum* represents certainly another genus, possibly more primitive than *Hordeum*.

## INTRODUCTION

Chromosome morphology has proved a valuable aid in showing the degree of relationships between species or populations of Gramineae (Morrison 1959). The karyotype analysis, though does not by itself indicate any definite phylogenetic affinity, it can fairly set up definite groups of species. Besides, combined with morphological or biochemical data, chromosome morphology increases the possibility of a more accurate identification of species relationships (Καράταγλης 1973, Booth and Richards 1978, Bothmer and Jacobsen 1979).

With regards to genus *Hordeum* L. the majority of work referred

to chromosome morphology have mostly been confined to common barley, because of its significance as a crop plant (Levitsky 1931, Hagger and Tjio 1952, Morrison 1959, Kasha and Noda 1979), with fewer reports about wild species (Chin 1941, Covas 1949, Oinuma 1952). However, no other cereal has so many wild relatives as barley (Rajhathy et al. 1964).

Morrison (1959) first gave considerable detail, by studying chromosome morphology in 16 *Hordeum* species, distinguished in seven groups with evident relationships. More recently Richards and Booth (1976) and Booth and Richards (1978) using both karyomorphological and biochemical methods of study gave sound evidence of evolutionary trends in *Hordeum murinum* group.

*Taeniatherum*, although defined as a separate genus by Nevski (1934) has for long by many taxonomists been given specific rank in the genus *Hordeum* (*H. asperum*, *H. crinitum*). Reports concerning any case of *Taeniatherum* karyotype have not so far been met in bibliography. Even chromosome numbers, by contrast to other genera of Gramineae are scarcely referred, and only by Griffée (1927) and Hunziker (1954), (Love and Love 1961).

In later years, the interest on wild species of *Hordeum* was revived. Within a project involving an investigation on world-wide scale (Bothmer and Jacobsen 1978) chromosome banding patterns in cultivated and wild barleys were defined (Vosa 1976, Linde-Laursen et al. 1979).

A project on native taxa of Gramineae, is in currency at our Department of Botany. Within it, Symeonidis (in publication) using electrophoretic analysis of various enzyme systems made essential steps towards identifying intraspecific, interspecific and intergeneric variation in local representatives of *Hordeum* and *Taeniatherum* species. Among the materials used, we selected some diploid species of *Hordeum* and *Taeniatherum*, in order to study their chromosome morphology and determine the corresponding karyotypes. The exact materials were chosen on the basis 1) of ploidy level (all diploids) and 2) of geographical limitation (all found in biotopes of Northern Greece).

The paper aims at contributing to the completer knowledge of Greek wild taxa of Gramineae.

#### MATERIALS AND METHODS

Two diploid species of the genus *Hordeum* were used, and namely *H. marinum* and *H. hystrix*. Both belong to *H. marinum* complex of

Trichostachys section. Besides, in the present study, one form of *H. marinum*, differing by only minor characters (ear hairiness) from the typical one has been included. One species of the genus *Taeniatherum*, concretely *T. caput-medusae*, completes the diploid taxa used here.

All materials represent local Greek populations collected during 1976-79, by the one of the authors (Symeonidis) in different areas of Macedonia.

1. *Hordeum marinum* Huds. (*H. maritimum* Stokes)

Site of collection: Kalohori, on the west of Thessaloniki.

2. *Hordeum marinum* I (with hairs in lemmas)

Site of collection: Georgiki Scholi, on the east of Thessaloniki.

3. *Hordeum hystrix* Roth (*H. maritimum* subsp. *gussoneanum* (Parl.) Asch. and Graebn., *H. geniculatum* All.)

Site of collection: Ptolemais.

4. *Taeniatherum caput-medusae* (L.) Nevski. (*Elymus caput-medusae* L., *Hordeum asperum* (Sim.) Degen, *H. crinitum* (Shreb.) Desf., *T. asperum* (Sim.) Nevski, *T. crinitum* (Shreb.) Nevski)

Site of collection: Mount Chortiatis.

Microscopic slides were prepared and the observations carried out on mitotic metaphase cells of root-tips. The usual Feulgen technique of staining and squashing was applied (Riley et al. 1958, Coucoli and Karataglis 1973, Καράταγλης 1973).

Good metaphase cells that were observed to have a similar degree of contraction and separately recognizable chromosomes were selected and immediately photographed. Photographs were also taken of a micrometer at the same power. The chromosome lengths were determined with a divider and measured on a 0.2mm scale and then converted to microns by comparison of scale with the photo of the micrometer at the same enlargement as the photo of the chromosomes (Joseph and Bouwkamp 1978).

The mean of the measurements of 10 cells from each material were taken (5 cells in *marinum* I). Total chromosome size, arm lengths and length of satellites (in SAT pairs) were calculated and karyotypes established by using quantitative and qualitative indices. More concretely: centromere class was defined by using the arm ratio (short/long arm) as suggested by Shrivastava et al. (1973) in the following way: median (M) = 1.00 - 0.76, submedian (SM) = 0.75 - 0.51 and sub-terminal (ST) = 0.50 - 0.26. Karyological terminology was supplemented according to the terms applied by Levan et al. (1965) and Κουκόλη

(1963). Relative length % (chromosome length X 100 / total chromosome length) was also calculated. In the idiograms the chromosomes were put in decreasing values of length.

## RESULTS

### *A. Hordeum marinum*

The karyotype of *H. marinum* was observed to contain the following synthesis of chromosome members (Table Ia, Figs. 1a, 2a).

1. One pair of satellited chromosomes, approximating the lowest threshold of SM class (a. r. 0.52, Fig. 1a, No. 4).

2. Two pairs of mesocentric (M class), the one among the longest, the other one of the shortest in the complement (2nd and 6th in the idiogram respectively).

3. One pair of strongly heterobrachial, acrocentric chromosomes (ST class), the shortest member of the set (No. 7).

4. Three pairs of submesocentric chromosomes, with arm ratio falling in SM class and representing index values 0.69, 0.65 and 0.69 respectively. (Table III, Nos. 1, 3 and 5 in the idiogram).

The chromosomes of this particular species were found to have small absolute size, ranging from  $5.90\mu\text{m}$  up to  $4.32\mu\text{m}$  (mean values).

According to the data presented in Table I, the variation in chromosome size of the different members of the complement appeared rather low, i.e. the relative length of the longest pair represents 16.40% of the total chromosome length, whereas that of the shortest pair corresponds to 12.01%. Or, the shortest chromosome is equal to 73.2% of the longest one.

The numbering, as well as the arrangement of chromosomes in order of decreasing length should be considered partly conventional, because the estimation of standard deviation (S) showed no significant differences between the length of most SM pairs, which proved to be overlapping in size. Thus, the constant chromosome markers are apparently: a) The presence of a satellited pair b) the existence of the two isobrachial pairs, significantly different in length and c) the appearance of one pair with subterminal centromere. The satellite size represents less than half the length of the short arm (ca. 40%).

By comparing the present results with the data given by Morrison for *H. marinum* (without numerical indices) a striking simi-

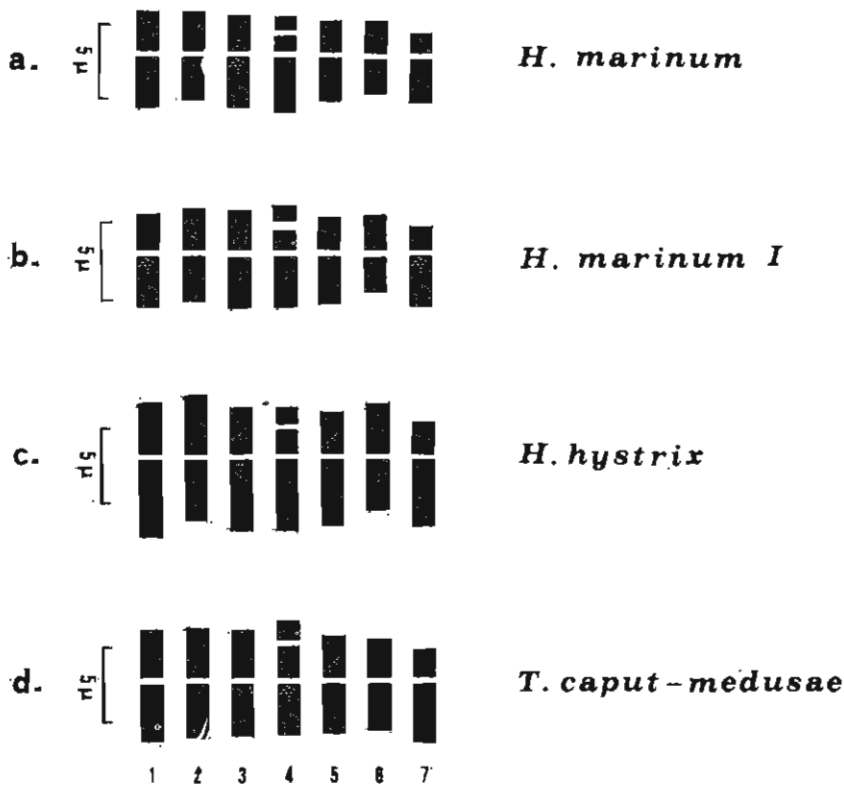


Fig. 1. Idiograms of the four karyotypes.

larity was noticed, concerning the morphological features of the chromosomes, in particular centromere class, yet in our material absolute size seemed to be more limited.

#### B. *Hordeum marinum I*

There was recognized a sound analogy to the karyotype of the previous species (Table Ib, Figs. 1b, 2b). The qualitative distribution of the chromosomes and the values of arm ratios are almost identical. The decreasing order of the size appeared also very similar, with the exception of the chromosomes Nos. 6 and 7 which showed a reversed relation. However, being given that these two members of the complement differ essentially in centromere class, the overlapping of size cannot

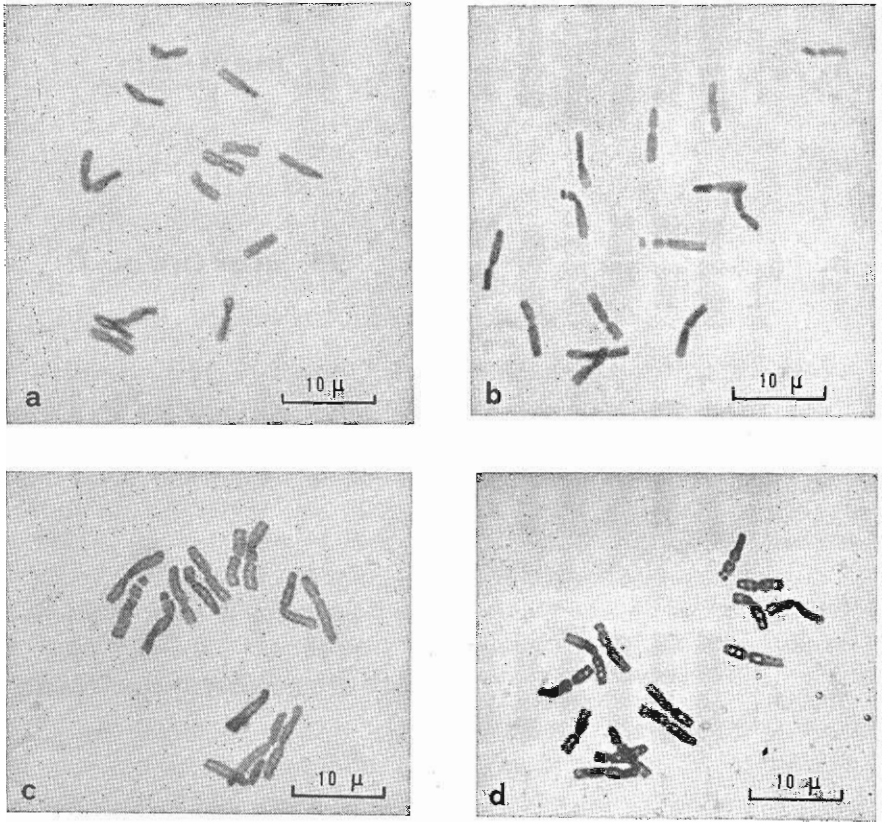


Fig. 2. Metaphase cells from root-tips of the four materials. a *H. marinum*, b *H. marinum* I, c *H. hystrix*, d *T. caput-medusae*.

cause any confusion. The relative length ranges between the same values and the shortest pair represents 72.2% of the longest one. The satellite length corresponds to 36.9% of the proximal short arm. The SAT pair falls in the same SM class, manifesting also the lowest value of arm ratio (0.62), comparatively to the rest SM members of the genome.

### C. *Hordeum hystrix*

The karyotype of this material appeared qualitatively similar, but quantitatively different from *H. marinum*. The absolute length values

of the chromosomes proved higher, ranging between  $8.36\mu\text{m}$  and  $6.20\mu\text{m}$  (Table IIa, Figs. 1c, 2c). Arm ratios were found to fall within the same centromere classes with the corresponding values of *H. marinum* chromosomes (Table III). The shortest member is equal to 73.8% the size of the longest one. SAT pair seems to be typically SM, representing the most heterobrachial member among SM chromosomes of the complement. The length of satellite was measured to possess 41.8% the size of the proximal arm. The deviation in length of the particular chromosomes proved more intensive, something rather expected with increased chromosome size.

Consequently, the three materials (*H. marinum*, *H. marinum* I and *H. hystrix*) showed evident qualitative similarity of karyotypes, *hystrix* being recognizable by only different absolute values of chromosome length.

#### *D. Taeniatherum caput-medusae*

A soundly different karyotype was observed in this species, which can be defined by the following characteristics.

(a) The variation of size proved more restricted, the shortest member representing 76.2% of the longest one, and the relative length ranging from 15.96% to 12.17% of the total chromosome length (Table IIb, Figs. 1d and 2d).

(b) Almost all members of the genome were recognized with less unequal chromosome arms. Six of them belong to the M centromere class among which only two approximate the mean values of 0.80 and 0.79 arm ratio respectively (Table III, fig. 1d, Nos. 1, 5). It is in the M class that the SAT pair belongs too, a feature quite distinctly separating this karyotype from those of the previous species. Satellite itself possesses 35.7% of the short proximal arm.

(c) One pair with subterminal position of centromere was constantly present which seems to be a typical index for all materials studied in this work.

(d) The decreasing order of size is more conventional, since it makes difficult to distinguish between the 2nd and 3rd chromosome pair as well as between the 5th and 6th members of the set. The only clearly recognizable chromosomes are the satellited pair and the ST pair.

## DISCUSSION

Morphological characters, as a means of classifying grass species, have a limited application in *Hordeum* genus, because spike features are useful to delimit species groups rather than single species. In other words, distantly related species are sometimes quite similar, whereas considerable morphological variability is often displayed in conspecific populations (Rajhathy et al. 1964).

The fact resulted in a great deal of confusion in the classification of *Hordeum* species. Some taxa have been described either as subspecies, or as species belonging to a broader complex, that is to a species taken in "sensu lato". This case holds for *Hordeum marinum* complex. On the other hand, *Taeniatherum* was ranked in the past by many authors in the genus *Hordeum* (*H. asperum* or *H. crinitum*) but in later years (Flora Europaea, Vol. 5, Gramineae, pp. 205-206) botanists returned to Nevski's aspect (1934), who accepted that as a distinctly separated genus, due to floral construction (number of spikelets per node). In addition, Baum (1978a, 1978b, 1978c) by using a numerical method of clustering, placed *Taeniatherum* in a different, not overlapping with *Hordeum*, cluster of taxa.

The establishment of karyotypes gives evidence about the constancy of chromosome morphology and permits justifiable comparison between or within species as well as, between species belonging to different genera. In karyotypical analysis 5 parameters can basically be applicable: (a) absolute size of chromosomes, (b) centromere position, (c) relative length of chromosomes, (d) basic chromosome number, and (e) number and position of secondary constriction (Stebbins 1971). All material studied in the present work was diploid ( $2n = 2x = 14$ ) with symmetrical karyotypes. *H. marinum* of our collection was similar karyomorphologically with the material used by Morrison in 1959 (origin: Botanic Garden of Uppsala), as regards both centromere class and variation of chromosome length between the members of the complement. The slightly higher values of chromosome length at least to the point they could be calculated from the photos in Morrison's paper (no numerical data) may be due chiefly to the different origin. *H. marinum* I showed the same karyotypical features with the former concerning the prominent size of the longest SM chromosome, (No. 1) the distinction between the 2nd and the 3rd chromosome pair by arm ratio and not by size, and the separation of the 6th and 7th pairs also by centromere class (overlapping in length). Total chromosome length



reaches almost the same values, whereas the distribution of relative chromosome length follows a similar variation. The results suggested no karyotypical differences, qualitative or quantitative, between the two forms of *H. marinum* which might reflect the morphological differences that forced us to consider them as distinct taxa. The close relationships between the two above types was defined also by analysing the isoenzymic pattern of certain systems (Symeonidis, in publication) which gave evidence of an essential number of common electrophoretic zones, identified by using the hypergeometric method.

The karyotypical distance of *H. hystrix* from *H. marinum* is based on the observed definite differences in chromosome size. However, a significant degree of resemblance regarding the qualitative characteristics of chromosome morphology was recognized. Centromere class was found almost identical. Besides, arm ratio of SAT pair appeared again as an indicator of extreme SM pair. The findings suggest that the two species, *H. marinum* and *H. hystrix* both belonging to the *marinum* group, sensu lato, might be considered to be conspecific (Morrison 1959, Rajhathy et al. 1964). Yet, we must admit that the present data cannot lead us to overlook Covas's view (1949) according to which only cytogenetic evidence can decide whether two entities are or are not conspecific.

The karyotype of *Taeniatherum caput-medusae* proved to deviate markedly from those found in *marinum* group. It was recognized even more symmetrical, with predominance of mesocentric chromosomes and lower rate between the shortest and the longest chromosome of the genome. Since *Taeniatherum* is a restricted genus of S. Europe, and only a variant from Krym (*T. crinitum*) which does not seem to merit specific rank is referred (in Flora Europaea), it could possibly be considered a rather primitive genus, on the assumption that evolution tends towards increasing of karyotypical asymmetry (Levitsky 1931). Irrespective of this, the morphology of chromosomes as defined in this study corroborates the position of *Taeniatherum* as a distinct genus, different from *Hordeum*. Recent data provided by Symeonidis (in publication), who established enzymic ratios to determine phylogenetic relationships in the same materials, confirm even strongly the aspect that *Taeniatherum* is quite remote comparatively to the diploid *marinum* species.

TABLE I  
Total chromosome length, arm length, arm ratio, and relative length in the species

Chrom. pairs	(a) <i>H. marinum</i>					(b) <i>H. marinum</i> I				
	Length in microns					Length in microns				
	total chrom. length	short arm	long arm	arm ratio	relat. length	total chrom. length	short arm	long arm	arm ratio	relat. length
1	5,90±0,80	2,42	3,48	0,69	16,40	6,10±0,56	2,42	3,68	0,65	16,24
2	5,62±0,46	2,78	2,84	0,92	15,55	5,74±0,25	2,74	3,00	0,91	15,25
3	5,56±0,38	2,23	3,33	0,65	15,45	6,03±0,61	2,55	3,48	0,73	16,02
4	5,42±0,18	1,86*	3,56	0,52	14,35	5,60±0,28	2,17*	3,43	0,62	14,88
5	5,02±0,28	2,06	2,96	0,69	13,95	5,13±0,37	2,07	3,06	0,67	13,63
6	4,42±0,28	2,15	2,27	0,94	12,29	4,40±0,42	2,09	2,31	0,90	11,71
7	4,32±0,46	1,38	2,94	0,46	12,01	4,63±0,23	1,49	3,14	0,47	12,30

total genome length 36,26

\*sat length 0,74

total genome length 37,63

\*sat length 0,80

TABLE II  
Total chromosome length, arm length, arm ratio, and relative length in the species  
(a) *H. hystrix* (b) *T. caput-medusae*

Chrom. pairs	(a) Length in microns				(b) Length in microns				relat. length
	total chrom. length	short arm	long arm	arm ratio	total chrom. length	short arm	long arm	arm ratio	
1	8,36±0,72	3,33	5,03	0,66	6,68±0,36	2,97	3,71	0,80	15,96
2	7,68±0,16	3,75	3,93	0,95	6,50±0,55	3,19	3,31	0,94	15,53
3	7,64±0,71	3,06	4,58	0,63	6,34±0,28	3,07	3,27	0,92	15,13
4	7,22±0,46	2,58*	4,64	0,56	5,96±0,62	2,80	3,16	0,88	14,23
5	7,20±0,13	2,76	4,44	0,62	5,78±0,36	2,56	3,22	0,79	13,80
6	6,26±0,48	3,42	3,14	0,96	5,40±0,60	2,31	2,79	0,82	12,17
7	6,20±0,70	4,95	4,25	0,45	5,52±0,46	4,74	3,78	0,46	13,18
	total genome length 50,56				total genome length 44,88				
	* sat length 1,08				* sat length 1,00				

TABLE III  
Arm ratios of the materials studied

Chrom. pairs	1	2	3	4	5	6	7
a							
<i>H. marinum</i>	0.69 SM	0.92 M	0.65 SM	0.52 SAT	0.69 SM	0.94 M	0.46 ST
b							
<i>H. marinum I</i>	0.65 SM	0.91 M	0.73 SM	0.62 SAT	0.67 SM	0.90 M	0.47 ST
c							
<i>H. hystrix</i>	0.66 SM	0.95 M	0.63 SM	0.56 SAT	0.62 SM	0.96 M	0.45 ST
d							
<i>T. caput - medusae</i>	0.80 M	0.94 M	0.92 M	0.88 SAT	0.79 M	0.82 M	0.46 ST

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ΠΕΡΙΛΗΨΗ

ΚΑΡΥΟΤΥΠΙΚΗ ΑΝΑΛΥΣΗ ΟΡΙΣΜΕΝΩΝ ΑΥΤΟΦΥΩΝ  
ΕΛΛΗΝΙΚΩΝ ΕΙΔΩΝ HORDEUM (ΟΜΑΔΑ MARINUM) ΚΑΙ  
TAENIATHERUM

Υπό

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Καθορίσθηκαν οι καρυότυποι σε τέσσερα διπλοειδή αυτοφυή είδη Gramineae του Βορειοελλαδικού χώρου. Τα τρία ανήκουν στην ομάδα *Hordeum marinum* (ευρύτερη έννοια είδους), και είναι τα ακόλουθα: *H. marinum*, *H. marinum I* (μορφολογικός τύπος) και *H. hystrix*. Το τέταρτο ανήκει στο γένος *Taeniatherum* (*T. caput-medusae*). Οι καρυότυποι μελετήθηκαν ποιοτικά (μορφολογία χρωμοσωμάτων, θέση κεντρομέρους) και ποσοτικά (μήκος χρωμοσωμάτων, μήκος βραχιόνων, διακύμανση μεγεθών, σχετικά μεγέθη). Διαπιστώθηκε: 1) Πλήρης σχεδόν ταύτιση των καρυότυπων των *H. marinum* και *H. marinum I*, τόσο ως προς τη μορφολογία των χρωμοσωμάτων, όσο και ως προς τις τιμές και τη διακύμανση των μεγεθών τους (απόλυτα και σχετικά μεγέθη). 2) Σημαντική ποιοτική καρυοτυπική αναλογία μεταξύ του *H. hystrix* και των δύο τύπων του *marinum* ως προς τη χρωμοσωμική μορφολογία (SAT ζεύγος, λόγος βραχιόνων, κατανομή σχετικών μεγεθών) αλλά διαφορά ως προς τις απόλυτες τιμές του χρωμοσωμικού μεγέθους (μεγαλύτερες τιμές στο *H. hystrix*). 3) Διαφορετικός καρυότυπος στο *T. caput-medusae*, δηλ. συμμετρικότερος με πλειονότητα μεσοκεντρικών μελών και διαφορετική μορφολογία του SAT ζεύγους. Από τη μελέτη των καρυότυπων συμπεραίνεται και επιβεβαιώνεται ή εκδοχή ότι τα τρία πρώτα υλικά ανήκουν στην ίδια ομάδα (σύμπλοκο είδος), ενώ το *Taeniatherum* αντιπροσωπεύει άλλο γένος, πιθανόν πιο αρχέγονο από το *Hordeum*.