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# **Certification system for the quality of honey bee queens in Greece**

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# Product: honey bee queens

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Since beekeeping manipulations involve:

- Ü the trade of colonies in a large extent
- Ü migration of colonies in long distances and
- Ü commercial production and trade of queens.

✦ Honey bee biodiversity and special characteristics of local races are in jeopardy.



- ✦ No national or controlled breeding system exists in Greece . . . . .
- ✦ Introduction of foreign queens is allowed, as by EU Directive 91/174/CEE.
- ✦ Beekeepers' demand for mated queens is very high in a specific time of the year (between April and August). Therefore, newly mated queens are sold very quickly after the initiation of oviposition without allowing evaluation of their performance for a long period



# Product: honey bee queens

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In an attempt:

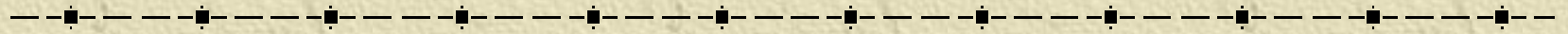
Ü to promote the use of local honey bee races.

Ü to identify the characteristics of each race or strain produced commercially and

Ü to inform beekeepers in order to use queens with better quality



# Product: honey bee queens



✦ We established an  
Accredited Certification System of  
Honey Bee Queens from Greece



# The first requirements

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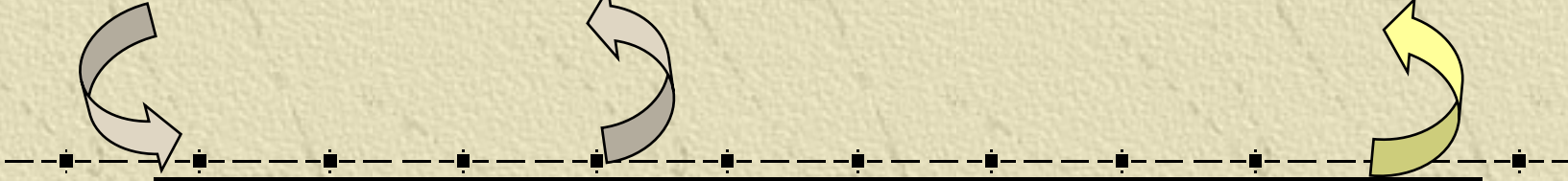
Accreditation has been given to:

1. a Certification Body for Honey Bee Queens (in the respect of testing genetic origin and queens' quality)-(ELOT EN/ISO 45011)-Laboratory of Agricultural Zoology and Entomology, A.U.A----M. Bouga)-
2. a Laboratory for Verification of honey Bee Queens Quality - (ELOT EN/ISO 17025)-Hellenic Institute of Apiculture, N.A.G.RE.F----F..Hatjina

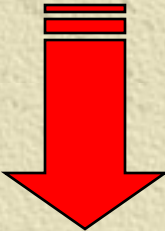


**Queen breeders**

**Ministry**



**The Certification Body**



**Accredited laboratories**



**Genetic origin**

**Queen quality**

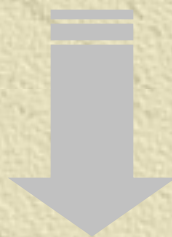


Queen breeders

Ministry



The Certification Body



Accredited laboratories



Genetic origin

Queen quality





# Queen quality

For the determination of the quality of young laying queens, the following parameters are measured:

- Ø Percentage of worker brood cells.
- Ø Percentage of empty brood cells.
- Ø No. of ovarioles.
- Ø Diameter of spermotheca.
- Ø Presence of Nosema spores.



# Procedure protocols

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All measurements are based on:

- ✦ International bibliography.
- ✦ Our personal data collected during the initialization of the quality system.



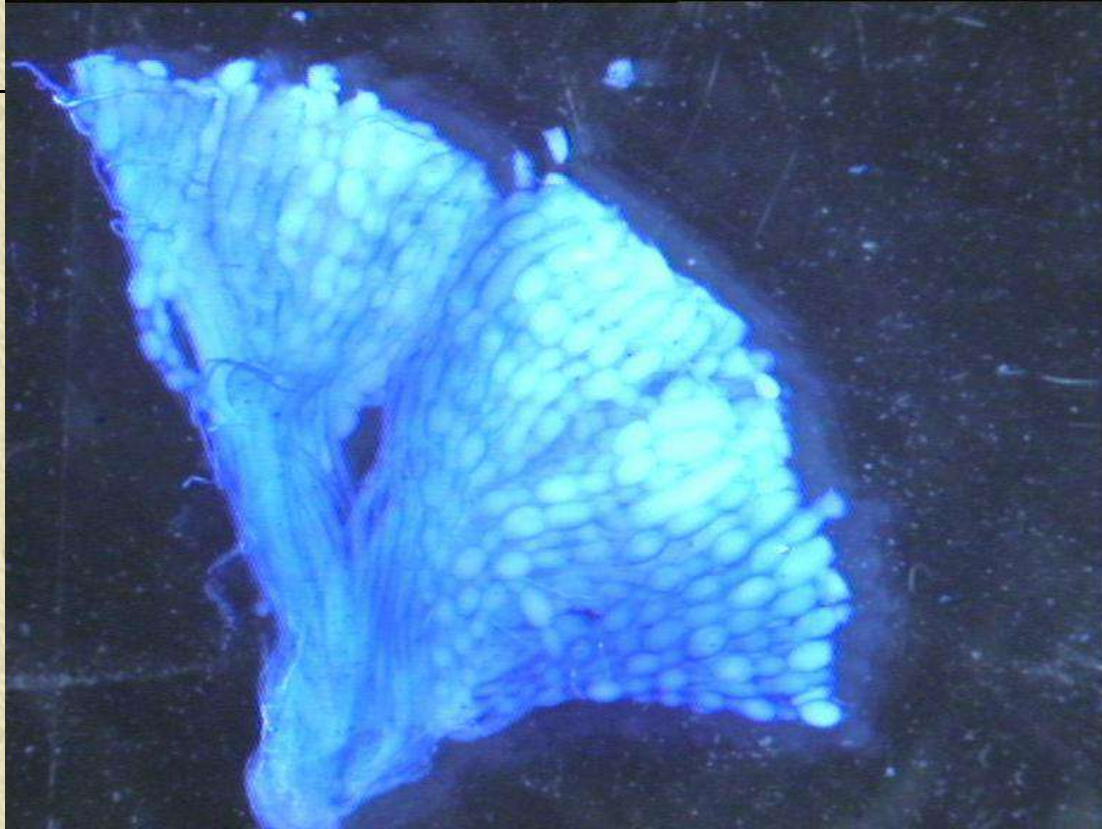
# Percentage of worker brood



Inside the circle of the brood area only  $< 10\%$  of cells are allowed to be empty and no cells are allowed to be drone cells



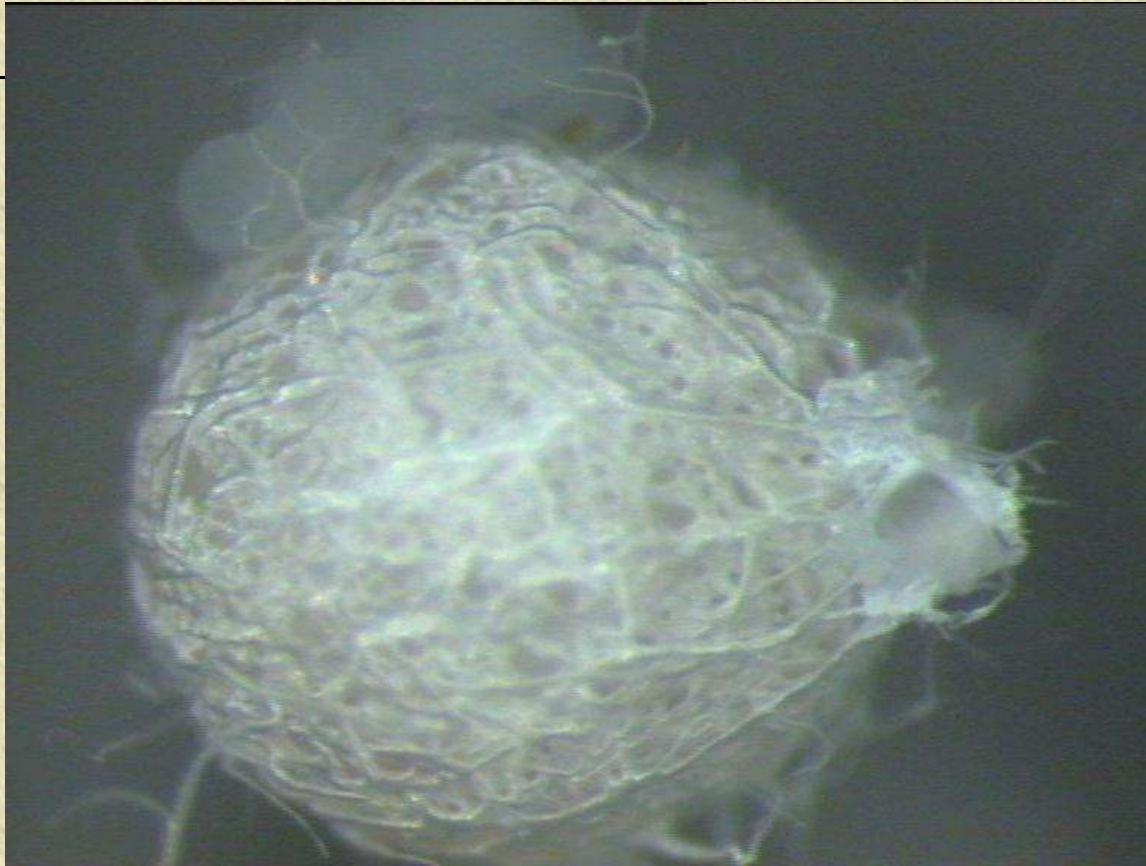
# Number of ovarioles



Every tested queen should have at least 130 ovarioles in each ovary



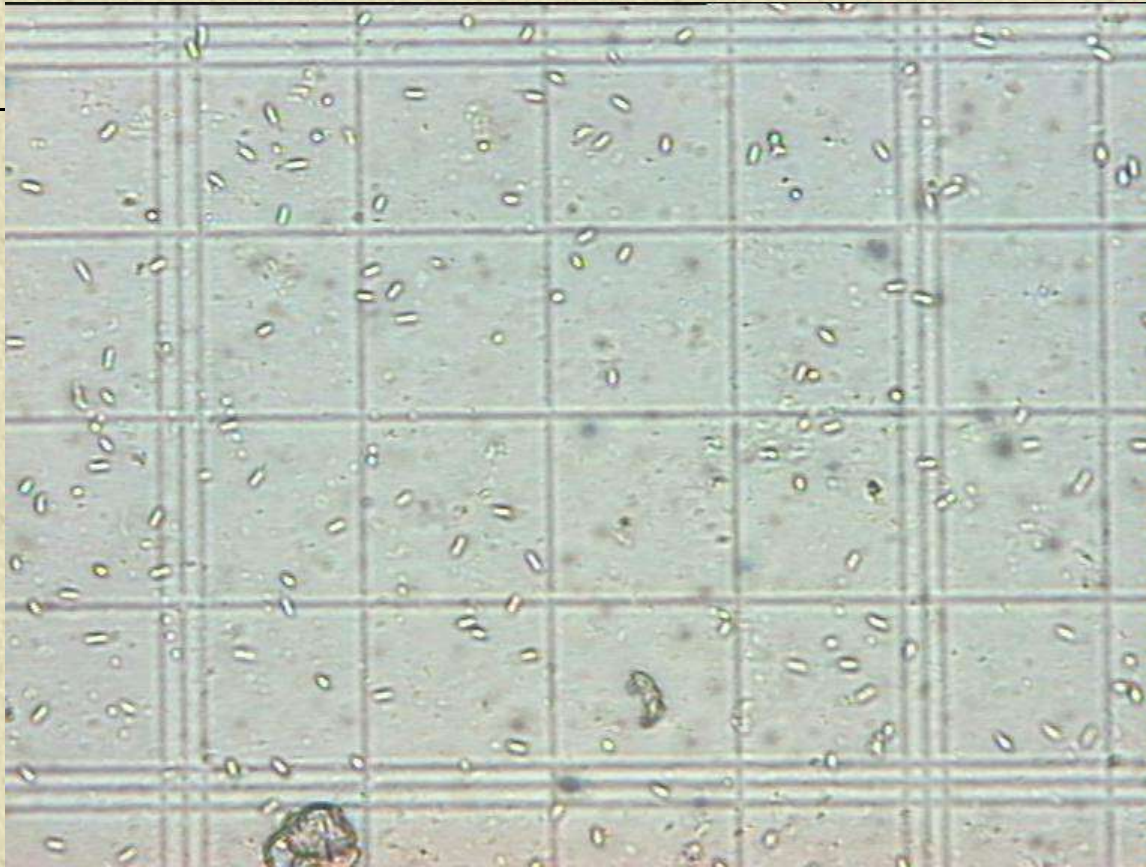
# Diameter of spermotheca



The diameter of spermotheca should be  $> 1,2$  mm



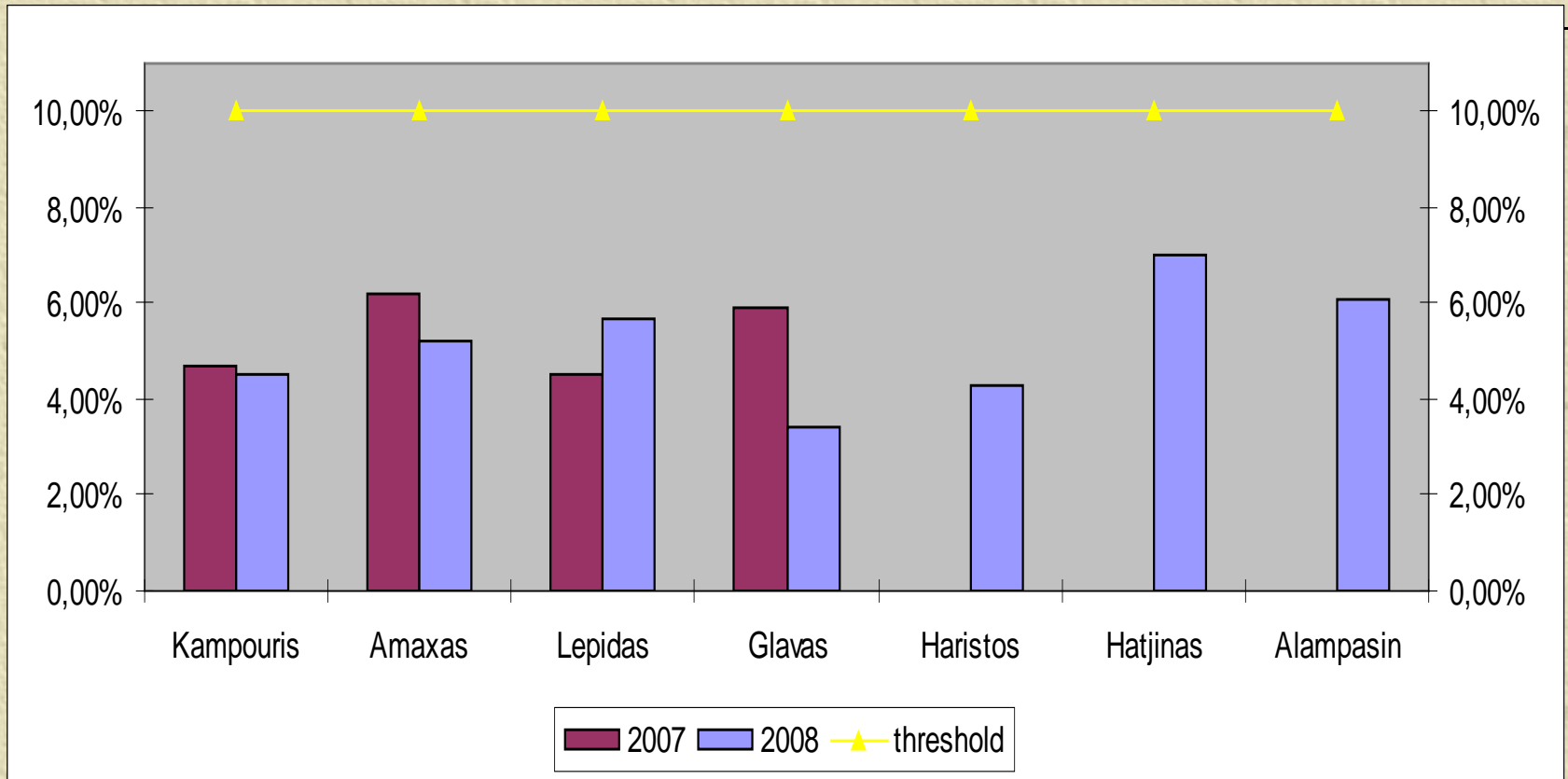
# Presence of Nosema spores



Nosema spores in the gut of the queen should not be  
> 500.000



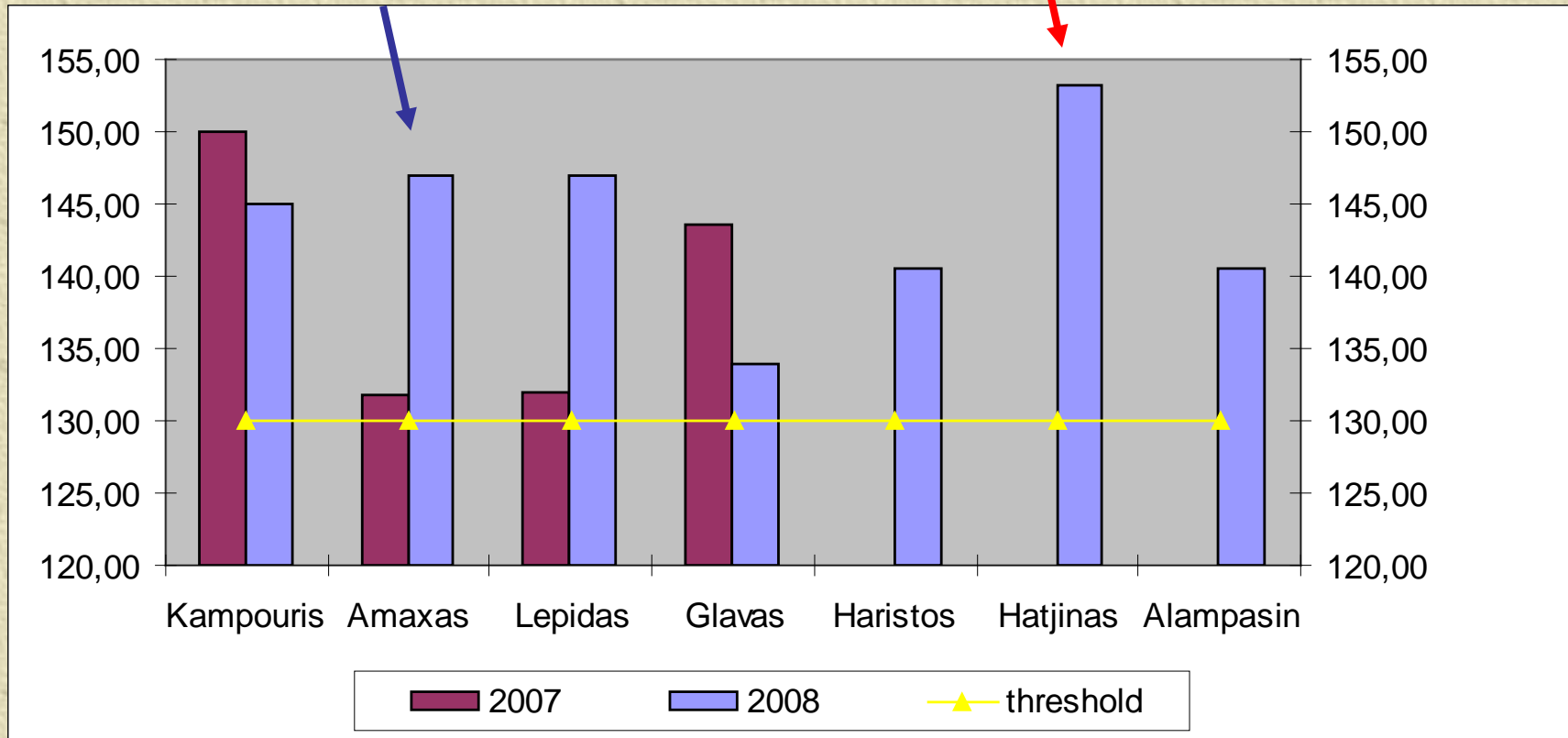
# Results



✦ Percentage of empty brood cells



# Results

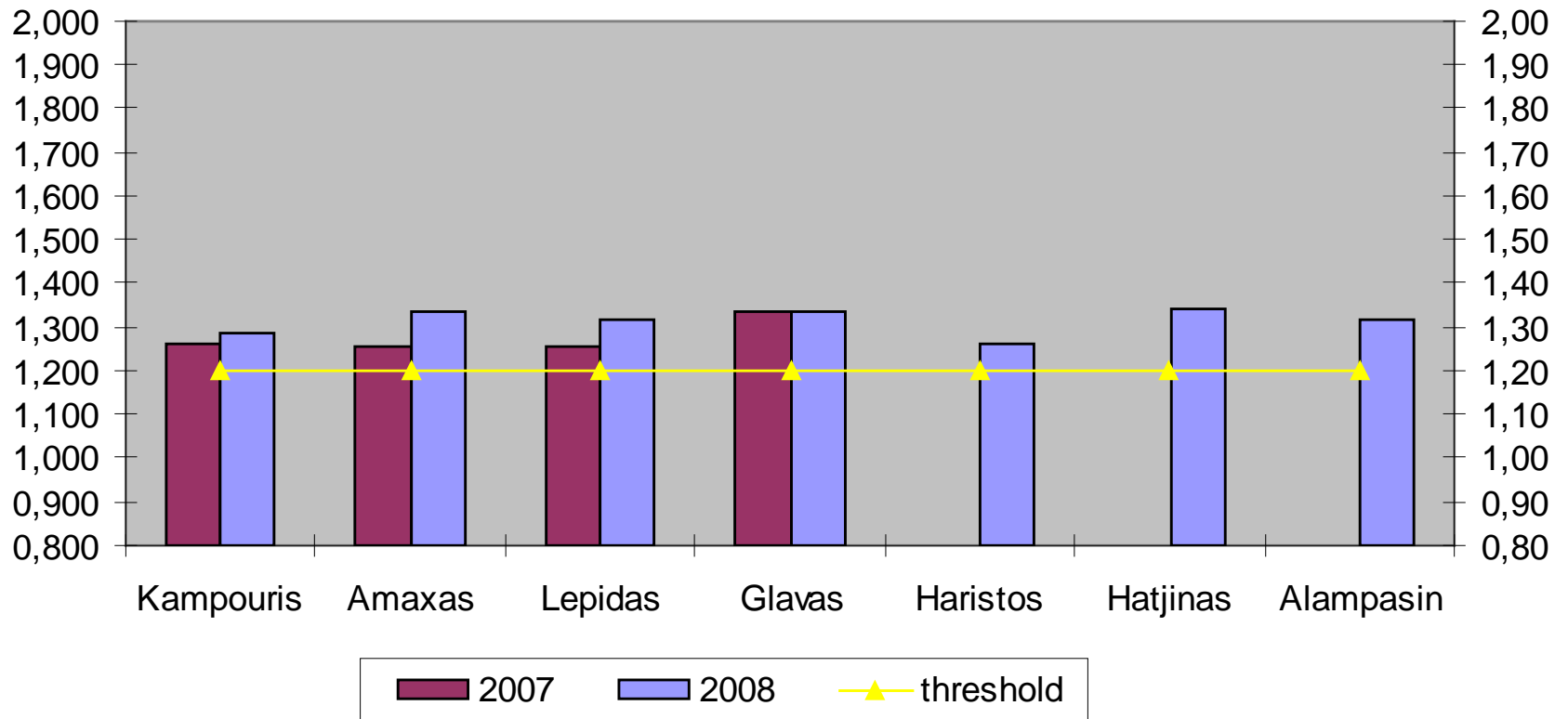


✧ Number of ovarioles





# Results



✦ Diameter of spermatheca



✦ Quality characteristics used:

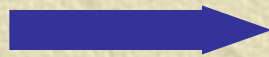


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According to the ones  
specified in the Certification procedure

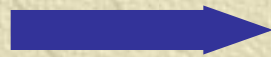
✦ Other important Quality characteristics



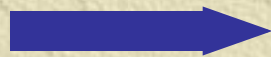
long term brood production



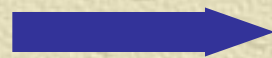
honey production



aggressiveness



low swarming tendency



sperm numbers



# Genetic origin of queens

- ✦ Mt-DNA analysis
- ✦ Sequencing of mtDNA gene segment CO I

Bouga *et al.*, (2005). *Apidologie*  
36:335–344

Bouga *et al.*, (2005). *Journal of Apicultural Research* 44 (4):  
195-196.

Bouga *et al.*, (2005). *Biochemical Genetics* 43:471-484.



# Materials and Methods

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- ∅ Extraction of total DNA according to the protocol of Hunt & Page (1992) after minor modifications  
(Bouga *et al.*, 2005)
- ∅ Amplification of gene segments using the PCR (Polymerase Chain Reaction)  
(Saiki *et al.*, 1988) method



# Materials and Methods

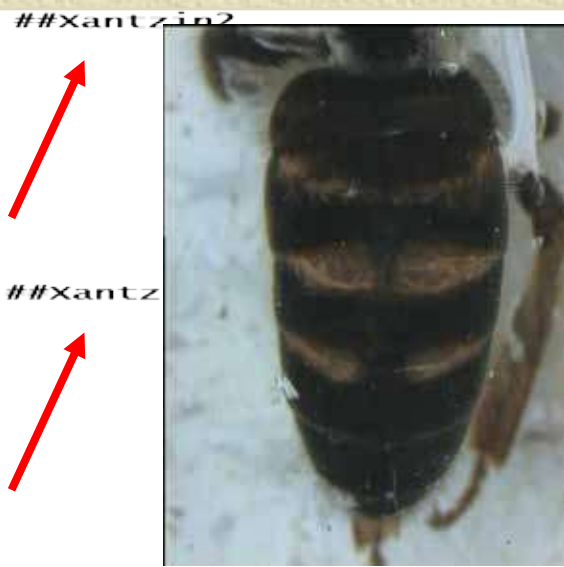
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One set of primers were used for the amplification of CO I (*Nielsen et al. 1999*):

5' GATTACTTCCTCCCTCATTA 3'  
3' AATAAGTCTGATAGGTCTAA 5'



# Results



BAS6-CO I



UPGMA dendrogram



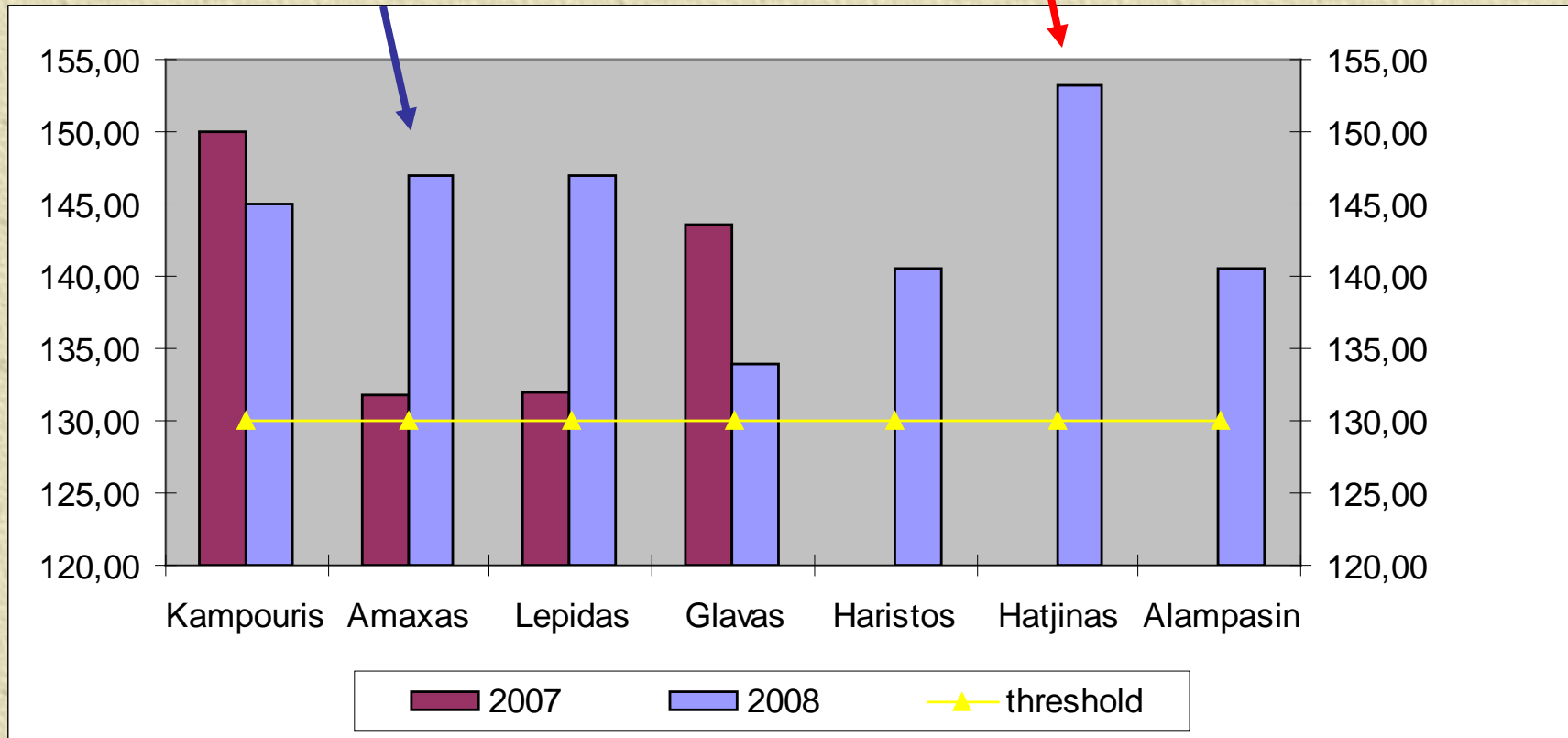
BAS4-CO I

BAS1-CO I

BAS5-CO I



# Results



✦ Number of ovarioles



# Procedure:

- ✦ The bee breeder sends an application to the Accredited bodies every month he produces queens
- ✦ The Accredited bodies decide when they will collect samples from the breeders. Sampling procedure is very strict
- ✦ All queens produced by these breeders must be marked with the specified colour by the Accredited bodies
- ✦ After all analysis, the two bodies produce a Certificate for each month with the characteristics of the queens of every month used for the analysis
- ✦ The accredited bodies charge all certification procedures