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ECOLOGICAL, ELECTROPHORETIC AND ELECTRON MICROSCOPE INVESTIGATIONS ON *SCHIZIDIUM PERPLEXUM* (ISOPODA) OF CRETE

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Introduction

The troglobitic isopod *Schizidium perplexum* (VANDEL, 1957) has been recorded from four caves in central-east Crete (Agia Paraskevi, Milatos, Peristera and Kronio) (Vandel 1957, Beron 1986). We recently found it in two more caves (Mikro Katofygi and Diktaio Antro) (map in fig. 3). All these caves are located considerably far from each other and at different altitudes (PLATAKIS 1975) (fig. 3) so that their isopod populations are geographically well separated.

The pressures for adaptation, mainly in the oligotrophic conditions of the caves, must be very strong. Thus the adaptations might have as a main direction the restriction of energy loss and the increase of the tactile and chemoreceptor sensitivity.

The present contribution deals with genetical and morphological aspects concerning the isopod populations of the caves Agia Paraskevi, Milatos, Peristera and Kronio. Some ecological data are also given.

Materials and methods

Few animals from each cave were collected and transferred to the laboratory alive. Five whole adult specimens from each cave were homogenized in a mortar. The extracts were electrophorized horizontally in 10% starch gels at 400 volts and 4^0C for 4 hours. The electrode buffer was 60mM lithium hydroxide – 0.3M boric acid. The gels were made with a mixture of 1% electrode buffer and 90% 30mM tris – 5mM citric acid; they were sliced and the inner surfaces were stained for: (1) esterases, with 5ml of 1% a-naphthyl acetate in 1:1 acetone and water and 150mg fast blue BB salt in 100ml 0.1M Na_2HPO_4 pH 6.4 and (2) peptidases, with 5mg L-leucyl-L-tyrosine, 1mg snake venom, 1mg peroxidase, 10mg carbazol (dissolved in 0.5ml NN-dimethylformamide) and 350mg molten agar in 50ml 25mM na_2HPO_4 pH 7.6.

Two male and two female adults from each cave were killed with 2-methyl-ether. The last three pairs of pereiopoda of the male individuals were removed in order to make the gonopods visible. After dehydration, critical-point drying and

coating with Au-Pt (300 Å), the specimens were studied in the scanning electron microscope.

All the ecological observations were made in the field.

Results and discussion

The patterns of 2-esterase and 1-peptidase isozymes differed among the caves (fig. 1). Esterase-1 appeared always monomorphic but with a slower allele dominating in Agia Paraskevi. Esterase-2 and peptidase appeared polymorphic with different allelic frequencies in each cave. These differences seem quite clear and are attributable to the genetic isolation of the caves. A more extensive study of these and other polymorphisms would be helpful in estimating the genetic distances among the populations and understanding the natural history of the caves.

Although the body size of adult individuals differed a little from cave to cave, the diagnostic characters were quite stable. SEM investigations showed two, more or less circular areas – one at each side of the head – which were smooth in contrast to their surroundings (arrows in fig. 2). This suggests that they are remnants of the original compound eyes. Below these areas, in the base of the cephalon, there were five reduced ommatidia two of which were hard to see (square in fig. 2). The number of ommatidia and their external structure differed among the caves as well as within the populations suggesting a gradual reduction of these organs towards total disappearance.

S. perplexum was found in the constant temperature, dark zone of the caves which is an extremly constant environment. Active animals were found simultaneously on the ground, inside the soil and under stones the whole year round suggesting that the constancy of the environment helps this species to live in various microhabitats, something that is not observed in non-troglobitic species.

The size of the populations is mainly determined by the food supply (bat guano), the size of the cave and the human influence (fig. 3).

The caves of Agia Paraskevi and Milatos are richer in bat guano so they are more diverse than the others and they support relatively large populations. The small size of Mikro Katofygi and the absence of inhabiting bats makes it less diverse. The caves Diktaio Antro and Kronio have been dramatically disturbed

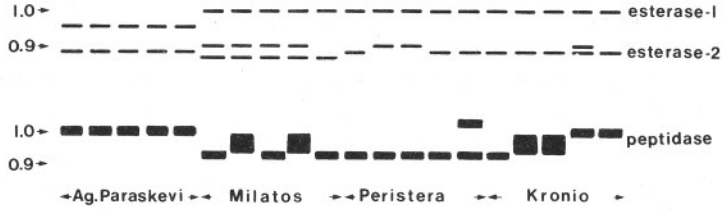

Fig. 1. Isozyme patterns of *S. perplexum* from different caves. The scale represents relative distances from the origin.

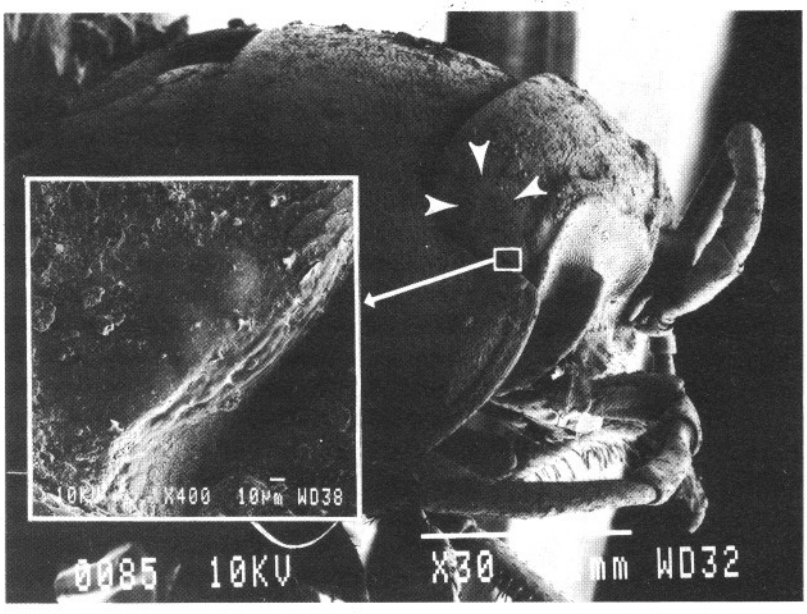

Fig. 2. SEM photograph showing the right side of the head of *S. perplexum*. The enlarged area shows the existing ommatidia.

by tourism. It seems that the *S. perplexum* populations in these two caves are about to become extinct.

Conclusions

Although the small populations did not allow us to study a large number of animals, certain interesting conclusions can be derived from this contribution.

The isopod populations are genetically isolated from each other and have developed different polymorphisms in at least two isozymes.

The majority of the primary ommatidia have disappeared and there is a tendency of disappearance of the few that remain. There no other strong morphological differences between the populations except a small one in body size.

The constancy and the strong similarities of the environments of all six caves suggest that the adaptive tendencies are quite the same, so at this level of the evolution of *S. perplexum,* speciation mechanisms are working very slowly.

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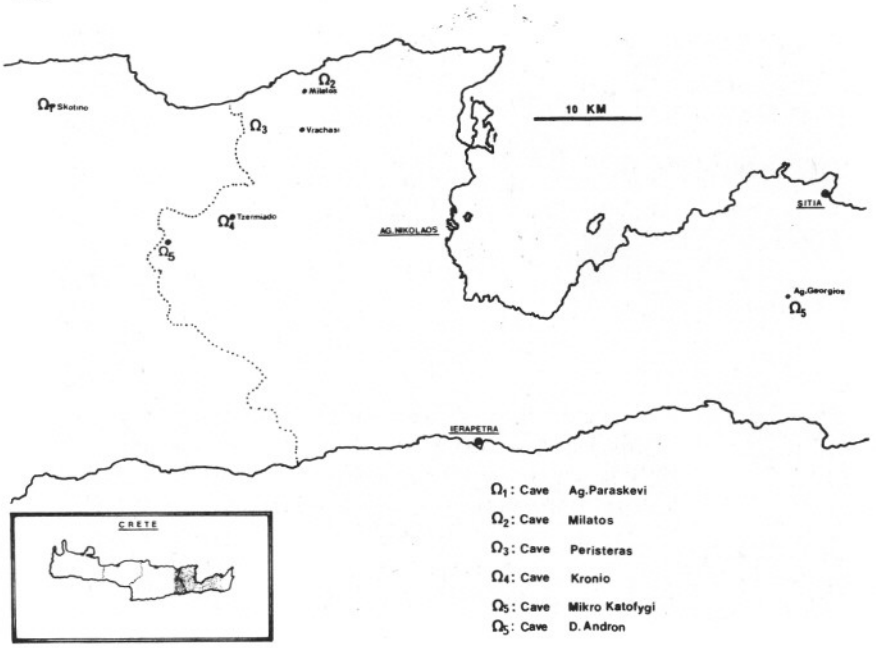

CAVES	ALTITUDE	LENGTH	TEMPERATURE	MOISTURE	SPECIES NR	POPULATION SIZE	GUANO
Ag. Paraskevi	230 m	160 m	12,2 °C	80-90 %	22	• • • • • • •	• • •
Milatou	70	70	15	70-80	16	• • • • • •	• • • • •
Peristeras	130	60	15	70-80	12	• • • •	• •
Kronio	912	30	16	70-80	5	• •	
Mikro Katofygi	460	25	15	70-80	4	•	
Diktaion Andron	1025	85	13	70-80	10	•	

Fig. 3. Map showing the location of the caves inhabited by *S. perplexum* and table showing some ecological characteristics.

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