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ELECTROPHORETIC INVESTIGATIONS ON TAXONOMY OF SOME SPECIES OF EPHEMEROPTERA FROM THE DUNAJEC BASIN (POLAND)

A. Kownacki and J. Starmach

Laboratory of Water Biology, Polish Academy of Sciences,
Slawkowska 17, 31-016 Kraków, Poland

Abstract. Taxonomy of aquatic insects, especially of their larvae, pupae or nymphs, is now very difficult. It happens often that the youngest stages of many species are so alike that sometimes it is impossible to determine them. To overcome these problems one of the most modern methods -biochemical systematics based on electrophoretic lipid separation was applied. The best results were obtained by using esterase enzymes. The following questions were asked: /a/ are the patterns of esterase separation the same during the whole species life; /b/ what the formula differences in closely related species; /c/ what are the differences between the specimens of particular genus. The mayfly larvae were investigated. They were chosen as being relatively easy to be morphologically distinguishable and have large biomass. The studies were carried out during one year on Baetis alpinus and Rhithrogena loyolea collected in Olczyski spring /Tatra National Park/; other species were found in Sucha Woda stream /1,000 m a.s.l./ /Baetis melanonyx/ and in Dunajec river /Baetis vardarensis, B. sinaicus, Oligoneuriella rhenana, Ephemerella ignita, Ecdyonurus sp./. These investigations proved high usefulness of electrophoretic method for mayfly determining.

Esterases, 7 spp., variability, biosystematics

Identification of preimaginal stages of insects, which constitute the main component of freshwater benthic invertebrates, is difficult because the taxonomy is based mainly on adult insects.

Mayflies belong to one of the well recognized groups of aquatic insects. Taxonomic features of nymphs allow easy separation at the generic level, however, it is much more difficult to identify the species precisely. Usually only the mature sta-

ges of nymphs are described. In the key for the genus Baetis Müller-Liebenau (1969) there is a passage which says: "The features characteristic for each species ..... are fully developed only in later stages of development and therefore small nymphs may be hard of impossible to identify". Therefore in hydrobiological papers species hard to separate are often treated together. E. g. Kownacka (1971) treated Baetis alpinus and B. melanonyx as one group. Further, authors presenting life cycles are not able to identify youngest stages, e.g. in Sowa (1975b) Rhithrogena semicolorata and R. diaphana nymphs up to 3 mm in size were not separated in a more precise way.

Another problem causing great difficulty is in defining the range of individual variation of species, especially of species which are identified using relatively subjective criteria, such as colour patterns, proportions, or size.

The aim of the present investigation is to describe a method which permits relatively quick separation of species that are hard to identify using morphological features.

Techniques of electrophoretic protein separation, described by Smithies (1955) and Hunter and Marker (1957), which brought about great changes in systematical investigations on plants, animals and man were chosen for this purpose. The number and pattern of bands of electrophoretically separated protein depends on its particular polymorphous form which is in directly related to the genotype. This permits one to distinguish between species even when very similar morphologically, and additionally between populations of the same species. Electrophoretic separation of insect protein was used by Townson (1969, 1982) and Bullini and Culuzzi (1972) to investigate genetic differences between mosquito species and their individual populations.

The enzyme esterase was chosen to investigate the differences between particular species of mayflies. Esterase influences various physiological functions of organisms (Burston 1962). It is a useful substance for systematic investigations because this protein is highly polymorphic acting in many cases even as an intraspecific indicator. Moreover, esterases are very stable in deep freeze storage (Nyman 1971), i. e. the frozen material remains in an unchanged state for a long time.

The present investigation attempts to answer the following questions:

(1) What are the differences in electrophoretic patterns among the representatives of particular species,

(2) What are the differences in patterns in closely related species,

(3) Are electrophoretic patterns the same in all stages throughout the whole life cycle?

MATERIAL AND METHODS

The investigation material was collected in streams and rivers of the Upper Dunajec River. Mayfly species composition and biology in this region is relatively well recognized, mainly due to papers by Sowa (1975a, b, 1979) and Kownacki (1980); this facilitated collection and identification of the material. In order to collect a sufficiently large amount of material, which had to be identified in vivo, the following dominant species were taken into consideration: Baetis alpinus, B. vardarensis, Rhithrogena loyolaea, Oligoneuriella rhenana, and Ephemerella ignita. In addition, B. melanonyx and B. sinaicus which are less numerous species but closely related with the previously mentioned species of the genus Baetis were selected. A separate problem was that of clasification of the relationship of larvae identified on the basis of body colour as Ecdyonurus forcipula (cf. Mikulski 1936). Sowa (1975a) reported that larvae of that colour are only a variety of E. venosus. Unfortunately it was not possible to collect a satisfactory amount of material of E. venosus coloured in the normal way and thus no comparison could be made between these two forms. In this study larvae of E. forcipula colour were treated as Ecdyonurus sp. In total eight species belonging to five genera were investigated.

Earlier investigations (Kownacki 1980) showed that in the spring sector of the Olczyski the mayfly fauna is represented by two species: Baetis alpinus and Rhithrogena loyolaea. Therefore, at this station nymphs of these species were collected at monthly intervals the whole year round, and were arranged in size groups. Other species were collected successively as they appeared at other stations in the catchment basin of the River Dunajec.

The mayfly nymphs collected were transported in vivo to the laboratory in Cracow and there identified and separated under 16x magnification. The material was frozen at - 20°C and subsequently homogenized with buffers 0.040 M tris and EDTA (hydroxymethyl aminomethan and ethylenediyminetetra-acetic acid) added in 2:1 proportion to the body weight of the larvae. The supernatant obtained after centrifuging was diluted 1:1 with distilled water placed on 10 % polyacrylamide gel.

Disc electrophoresis was carried out on polyacrylamide gel for 110 min. at a current intensity of 2mA per tube (6 mm in diameter) using the following buffers: 0.040 M tris and EDTA to the gel and 0.050 M H_3BO_3, and LiOH in vessels. After completing electrophoresis the non-specific esterases separated on gel were stained following Nyman (1970).

RESULTS AND DISCUSSION

Electrophoretic patterns of non-specific esterases separation of eight species of mayflies were presented in Fig. 1. Patterns and number of particular bands of esterase protein show relatively large genetic differences between the species especially

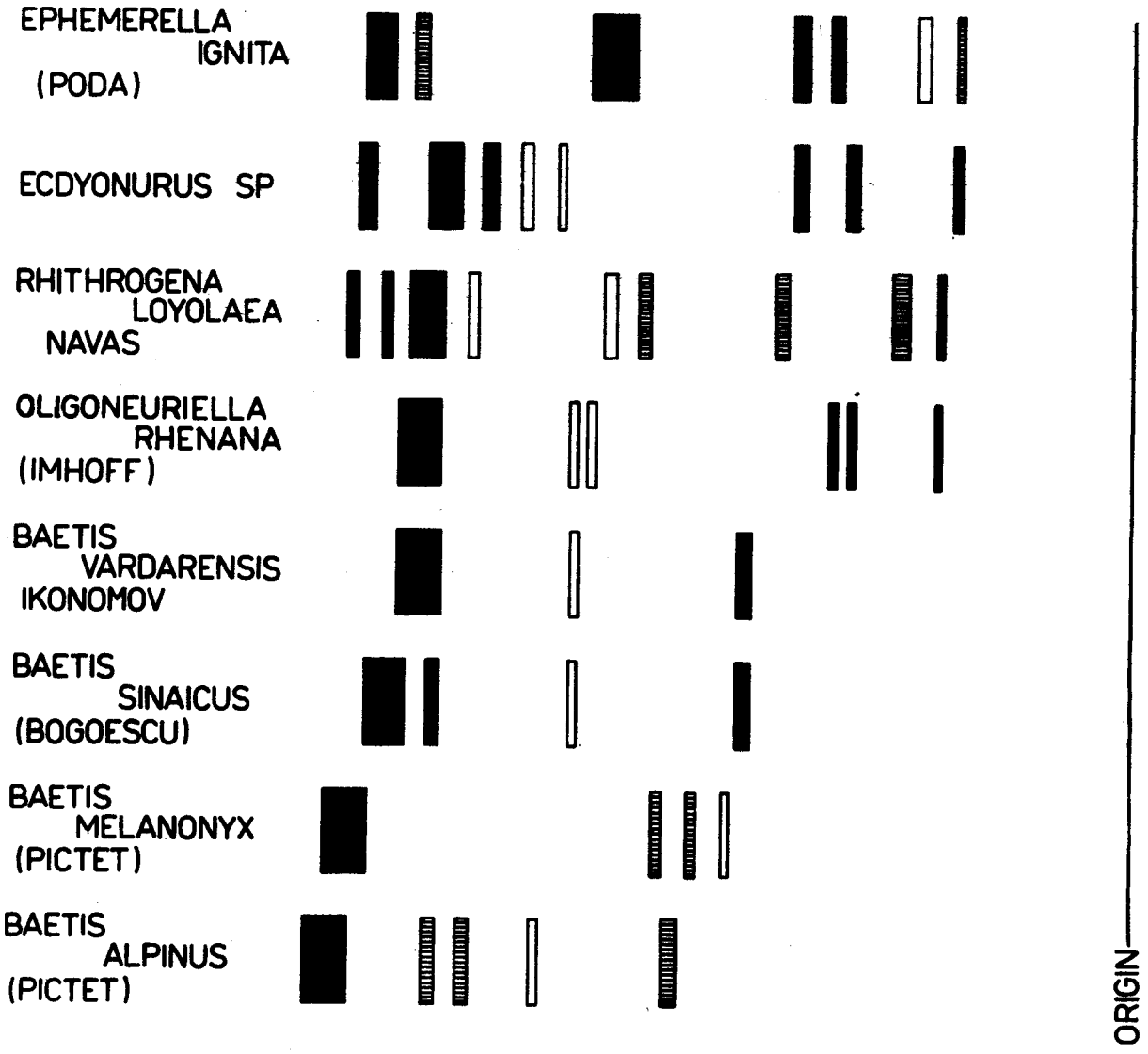

Fig. 1: Electrophoretic non-specific esterases separation of eight species of mayflies.

between representants of individual genera. This was expected because the investigated species represent the three super-families: Siphlonuroidea (Baetis), Heptagenioidea (Oligoneuriella, Ecdyonurus, Rhithrogena), and Leptophlebioidea (Ephemerella) (Landa 1976). Representatives of the genus Baetis differ most from the other groups. These species are characterized by highest speed of "slow" esterases. The species of the genus Baetis examined simultaneously have most similar patterns. This results at least partly from the fact that these are very closely related species, inhabiting very similar biotopes. In analyses of these electrophoretic patterns, however, a discrepancy was found in the representatives of the genus Baetis between traditional classification based on morphological features (Müller-Liebenau 1969) and the results obtained from electrophoresis. From among four species of the genus Baetis the most similar patterns are those of Baetis sinaicus and B. vardarensis. They differ only by one band and a small difference in the (speed) of "fast" esterases. Whereas, on the basis of morphological features these two species were included

in two different groups. Baetis vardarensis was included the lutheri group and Baetis sinaicus in the lapponicus group. For the species Baetis sinaicus even a separate genus Acentrella (Bogoescu 1931) was introduced initially. Whereas, species Baetis alpinus and B. melanonyx traditionally joined into one group and treated as one in a number of papers, have electrophoretic patterns indicating to a distant relationship.

— Nymphs of Baetis alpinus and Rhithrogena loyolaea were studied during the whole year in all instars. Other species, Baetis vardarensis, Oligoneuriella rhenana, Ephemerella ignita were also collected several times during the year at various stations, however not so systematically. It was found that the patterns of electrophoretic separation of esterases in mayflies do not change during their whole life cycle.

It may be eventually found that the electrophoretic separation of esterases, as with other groups of animals, is a very good indicator in differentiating species and should be included in investigations on mayfly systematics. This is a specially useful method for distinguishing between morphologically similar species.

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