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Isoenzymes and Taxonomy in Scandinavian Hydroids (Cnidaria, Campanulariidae)

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Acid phosphatases, esterases and leucine aminopeptidases were studied in *Campanularia integra, Clytia hemisphaerica* (the *johnstoni* form), *Laomedea flexuosa, Gonothyraea loveni, Obelia geniculata, O. longissima* and *O. dichotoma*, using polyacrylamide gel electrophoresis. Species-specific patterns were obtained, notably regarding acid phosphatase, despite strong individual variation. The electrophoretic results combined with differences in morphology and cnidom indicate that *O. longissima* and *O. dichotoma* are separate species. The acid phosphatase patterns of the two species and of *O. geniculata* showed basic similarities, supporting their assignment to a common genus. The profound differences in the acid phosphatase patterns of *C. integra* and *L. flexuosa*, may in conjunction with morphological distinction justify their assignment to separate genera. *C. integra* also included specimens of *caliculata* morphology: however, these did not deviate in their band patterns. Electrophoretograms of the typical *O. dichotoma* resembled those of the *dubia* and *plicata* forms. Moreover, *O. gelatinosa* with free *Obelia* medusae (sensu Hincks 1868) is suggested to be conspecific with *O. longissima*. The present study indicates that electrophoretic analyses, particularly of acid phosphatases presenting highly reproducible patterns, is a valuable complement to morphological characters when studying the taxonomy in hydroids.

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1. Introduction

The classification of hydroids belonging to Campanulariidae has been problematic and the generic value of the presence of medusae, medusoids or sessile gonophores as well as characters in the diaphragm and colony form have been the subject of much speculation by researchers working in this field. Moreover, the recognition of species has caused problems. Intraspecific variation was gradually discovered and the number of species belonging to Campanulariidae was recently greatly reduced (Millard 1966; Cornelius 1975). The different opinions about the validity of generic and specific characters, either due to genetic differences and/or the influences of environmental factors such as viscosity, salinity, temperature etc. of the sea water (Broch 1918; Mammen 1965; Millard 1966; Cornelius 1975) have resulted in continual changes in the taxonomic treatment of hydroids within the Campanulariidae. Therefore it seems desirable to employ new methods to supplement the morphological approach. Isoenzyme studies appear to offer one such possibility for further clarifying the relationship within this family.

In recent years reports have been published on the use of polyacrylamide gel electrophoresis (PAGE) to tackle taxonomic problems not only in vertebrates but in a great variety of other organisms ranging from bacteria (Nicolet *et al.* 1980) and fungi (Sorensson *et al.* 1971) to higher invertebrates (Bursey *et al.* 1980; Cantell & Gidholm 1977). Using electrophoresis, it is thus possible to compare isoenzyme patterns in and between the typical forms of the species.

The present investigation, making use of PAGE, was designed to study the taxonomy of Campanulariidae in Scandinavia in order to clarify inter- and intraspecific variation and reveal possible synonyms. For this purpose the electrophoretic band patterns produced by the enzymes acid phosphatase, esterase and leucine aminopeptidase was examined in a number of species.

2. Materials and methods

2.1. *Species investigated*

Hydroids from the Gullmar Fjord on the Swedish West coast (58°15′N, 11°28′E) were examined during the years 1970–1973. In spring 1970 investigations were made also of hydroids from the Bergen area on the Norwegian coast (60°23′N, 5°20′E). The animals were collected by SCUBA and free diving down to a depth of 25 m and by dredging on hard bottoms from 10 to 125 m. The classification of Campanulariidae proposed by Cornelius (1981) to the International Commission on Zoological Nomenclature is followed. The species investigated are accordingly placed in the genera *Campanularia* Lamarck, 1816, *Clytia* Lamouroux, 1812, *Laomedea* Lamouroux, 1812, *Gonothyraea* Allman, 1864, and *Obelia* Péron & Lesueur, 1810. The species and their morphological forms examined from different localities and substrates are given in Table I.

2.2. *Preparation of tissue extract*

Old hydroid colonies were often completely overgrown by various sessile organisms, mainly diatoms. Younger shoots and gonothecae were considerably cleaner. The diatom cover decreased with an increase in the depth from which the animals were collected. To check the possible contaminating effect of these epiorganisms or their influence on isoenzyme separation, parallel tests were performed in which strongly covered and clean materials were compared. To get material absolute free from epiorganisms, hydranths or gonophores were sometimes pinched out of their theca. Overgrown animals gave somewhat blurred electrophoretograms. Accordingly great care was taken to obtain as clean material as possible.

Probably due to intraspecific variation, pooled samples from different

Table I. *Survey of Campanulariidae examined electrophoretically*

	Gullmar Fjord, localities, substrates	Bergen area, localities, substrates	Different morphological forms
Campanularia hincksii Alder, 1856		On *Lophelia* corals, 100–125 m depth, Brattholmen	
Campanularia integra MacGillivray, 1842		On stalks of *Laminaria* spp., 20–25 m depth, Vattlestraumen	Colonies with thin-walled hydrotheca and spiral furred gonotheca. Colonies with thick-walled hydrotheca and smooth gonotheca, the *caliculata* form (sensu Hincks 1853)
Clytia hemisphaerica (L., 1767) of the *johnstoni* form (sensu Alder 1856)	On exposed shores on *Furcellaria fastigiata*, *Polysiphonia* spp., *Codium dichotomum*, 1–4 m depth, at Blåbergsholmen, Usholmen, and from the rocks at the Klubban Biological Station, and on *Delesseria sanguinea*, 10–20 m depth, at Smedjan		
Clytia hemisphaerica (L., 1767) of the *gracilis* form (sensu M. Sars 1851)	On ascidians, 15–40 m depth, at Hågarnskär	On hard bottoms, 20–25 m depth, Vattlestraumen, and on *Lophelia* corals, 100–125 m depth, Brattholmen	
Laomedea flexuosa (Alder, 1856)	On unexposed shores on *Fucus vesiculosus*, *Ascophyllym nodosum* and on bare rocks, 0.2–0.5 m depth, Blåbergsholmen, Långgap, Flatholmen, Usholmen	On *F. vesiculosus* and *A. nodosum*, 0.2–0.5 m depth, Raunefjord near to Espegrend	
Gonothyraea loveni (Allman, 1859)	On gently exposed and unexposed shores on *F. vesiculosus* and *A. nodosum*, 0.2–0.5 m depth, Kockholmarna and Långgap	On *F. vesiculosus*, 0.5–3 m depth, Raunefjord near to Espegrend	
Obelia geniculata (L., 1758)	On slightly exposed shores in the brown algae zone on *F. serratus*, *Laminaria saccharina*, *F. fastigiata* 0.5–4 m depth, Blåbergsholmen, Usholmen, Bonden, and on the rocks at Klubban Biological Station	On *F. serratus*, 0.5–4 m depth, Raunefjord near to Espegrend	Strongly branched and unbranched colonies. Colonies with different thickness of periderm
Obelia longisima (Pallas, 1766)	On exposed shores on rocks and shells in the littoral and on the red algae zone on *Polysiphonia* spp., *Ceramium* sp., *F. fastigiata*, *0.2–25 m depth, Blåbergsholmen, Usholmen, Gula skären*		Colonies with compound stems and branches, the *gelatinosa* form (sensu Hincks 1868) with free *Obelia medusae*. Colonies with uncompound stems and branches
Obelia dichotoma (L., 1758)	On *Chorda* spp., *Zostera marina*, *Polysiphonia* spp., *Ceramium* spp., *F. fastigiata* and ascidians, 2–40 m depth, Blåbergsholmen, Hågarnskär, Flatholmen, Långgap		Colonies with compound stems and branches, the *plicata* form (sensu Hincks 1868). Colonies with uncompound stems and branches. Hydrothecae with longitudinal striations from the incisions between the marginal teeth, the *dubia* form (sensu Nutting 1901)

colonies often presented diffuse enzyme patterns. Tests with the large colonies of *Laomedea flexuosa*, *Obelia geniculata*, *O. longissima* and *O. dichotoma* were performed on single specimens. Pooled samples composed entirely of either female, male or undifferentiated gonangia, or colonies without gonangia were analysed to test whether specific enzymes were coupled to the gonangia. Furthermore, samples of hydranths only or colonies without hydranths were also compared. The smallest samples analysed comprised material equivalent to the volume of about 50 hydranths.

Electrophoresis was conducted on fresh as well as frozen and thawed animals. Samples for freezing were packed in a Parafilm envelope together with a very small amount of sea water. The packages were carefully sealed with tape to prevent oxidation and drying of samples during storage. Each sample was treated in an ice-chilled all-glass hand homogenizer. To obtain a concentrated solution only a minute volume of distilled water was added, and the homogenizer was afterwards washed with a chilled, concentrated solution of saccharose and bromphenol blue (saccharose was used to increase the density of the sample and bromphenol blue as a dyemarker). The homogenate and washing solution were pooled and centrifuged at 15 000 *g* for 20 min at 0°C. The supernatant was used immediately for electrophoresis.

2.3. *Polyacrylamide gel electrophoresis and enzyme localization*

The electrophoretic technique described by Akroyd (1967) was used with the following modifications. The polymer solution was placed in the electrophoretic cell, and the edge of the cell immediately sealed with tape. Nitrogen gas was then gently blown over the solution to avoid oxidation during the polymerisation. After polymerisation, buffer was stratified on top of the gel to prevent drying. The samples were applied with Pasteur pipettes with long narrow tips. Folded filter papers, Munktell No. 205, were used as current bridges. The rubber tubes used as sample separators on top of the gel were not removed until the electrophoresis was completed. Electrophoresis was carried out at about +4°C.

Cyanogum 41 polyacrylamide gels were made according to Hjertén (1962); Hjertén *et al.* (1965) under the conditions listed below. Conventional staining methods for localization of the enzyme system were employed (see Pearse 1960; Latner & Skillen 1961; Coles 1969; Brewer & Sing 1970) with the mentioned modifications.

Acid and *alkaline phosphatases* were analysed in a continuous 0.1 M Tris-acetic buffer, pH 7.5, in 6 and 8% gels. The separations were run at

0.05–0.1 A and about 150 V for about 3 h. Staining: 50 mg sodium α-naphthyl phosphate, 50 mg Fast Garnet GBC salt/50 ml 0.05 M, pH 5.0, acetate buffer (acid phosphatases), 50 mg sodium α-naphthyl phosphate, 50 mg Fast Red TR salt/50 ml 0.1 M, pH 9.5, Tris-HCl buffer (alkaline phosphatases).

Esterases were analysed in a continuous 0.1 M Tris-acetic acid buffer, pH 8, in 10, 11 and 12% gels. The separations were run at 0.1 A and about 150 V for about 3 h. Staining: 20 mg α-naphthyl acetate, 0.5 ml acetone, 100 mg Fast Red TR salt/40 ml 0.2 M, pH 7.4 phosphate buffer.

Leucine aminopeptidases were analysed in a discontinuous 0.1 m Ashton buffer, pH 8.6 (Ashton & Braden 1961), in 6 and 8% gels. The separations were run at 0.05 A and 150 V for about 90 min. Staining: 20 mg L-leucyl-β-naphthylamid HCl, 50 mg Black K salt, 5 drops 10% $MgCl_2$/50 ml 0.2 M monobas natriumphosphate.

Malate dehydrogenases were separated in a continuous 0.1 M Tris-acetic buffer, pH 8, in a 6% gel, at 0.05 A and 110 V for about 90 min. Staining: 10 NAD, 5 mg MTT-tetrazolium, 5 mg phenazine methosulphate/50 ml 0.1 M Tris plus 1 g Malic acid sodium salt adjusted to pH 7.5 with HAc.

The number of samples analysed for each species ranged from 32 to over 160 for the acid phosphatases except for *Campanularia hincksii* and the *gracilis* form of *Clytia hemisphaerica* (5 and 8 samples, respectively). For esterases and leucine aminopeptidases the number of samples were around half of those for acid phosphatases.

Parallel runs with small and large amounts of the same sample and with diluted samples were performed. The incubation time was 15–20 h in order to give visible colour patterns even with low activity enzymes. During this period the gels were photographed by transmitted light, to obtain distinct colour patterns also from high activity enzymes. Distinct coloured parts of the electrophoretograms are here termed "bands" and broad, diffuse weakly coloured parts "zones", Bands and zones were numbered from the starting position. In order to facilitate the interpretation of the isoenzyme patterns, band profiles were arranged into groups, when the intraspecific variation was large.

3. Results

3.1. *General observations*

Acid phosphatases displayed highly reproducible electrophoretic band patterns. The electrophoretic separation of esterases and leucine aminopeptidases was less satisfactory, because in many runs the band patterns seemed incomplete. However, these three isoenzyme systems gave band patterns specific for each of the following species: *Campanularia integra*, *Clytia hemisphaerica* (the *johnstoni* form), *Laomedea flexuosa*, *Gonothyraea loveni*, *Obelia geniculata*, *O. longissima* and *O. dichotoma*. Due to their rarity *Campanularia hincksii* and *Clytia hemisphaerica* (the *gracilis* form) were not properly analysed. Strong individual variation was displayed in all thoroughly tested species. Concerning malate dehydrogenases and alkaline phosphatases, preliminary runs showed no bands at all, and these enzyme systems were therefore omitted.

The electrophoretic zymograms of hydroids studied from the Bergen area closely coincided with those obtained for corresponding animals from the Gullmar Fjord, although some minor differences were observed. No correlation was obvious between intraspecific variation and different habitats within each geographic area (see Table I).

In the comparative electrophoretogram studies of samples containing gonangia or polyps without gongangia some minor distinctions occurred in the phosphatase patterns of *L. flexuosa*, *O. geniculata* and *C. integra* and in the esterase and leucine aminopeptidase patterns of *L. flexuosa*. However, no dissimilarities were observed between extracts of only hydranths or of pedicels and stems without hydranths compared with samples of whole colonies without gonangia.

One round of freezing and thawing did not seem to affect the major acid phosphatase patterns. Good enzyme activity was achieved for this enzyme system with material stored frozen for up to two years. However, for esterases and leucine aminopeptidases obviously better results were obtained with fresh material.

3.2. *Acid phosphatases* (Fig. 1)

General similarities in acid phosphatase band patterns were found in the species studied. The patterns were made up of a number of slow migrant bands of varying intensity and a front of a strongly coloured, broad band or group of bands. Sometimes a few even faster, weak bands appeared. The migration rate of the strongly stained front was highest in *O. dichotoma* and gradually lower in *Cl. hemisphaerica* (the *johnstoni* and *gracilis* forms), *G. loveni*, *L. flexuosa*, *O. geniculata*, *O. longissima* and *C. integra*.

In addition to the low mobility of the front, *C. integra* was recognized by a broad, generally strong, single or divided band at or near the starting position (Fig. 2). Sometimes the faster band *7* was also apparent in samples from colonies.

The phosphatase zymograms of the *johnstoni* form of *Cl. hemisphaerica* and of *L. flexuosa* (Figs. 2, 3) were basically similar with two groups of slow bands in addition to the strong front band. The front of the *johnstoni* form was, however, much faster than the front of *L. flexuosa* (Fig. 3). Strong individual variation, including differences in both female and male gonangia, was manifest in *L. flexuosa*, and electrophoretograms from the Gullmar Fjord have been ranged into three groups (here termed) A, B and C, respectively. Band *5* (*B* in Fig. 3) was most common in extracts of male gonangia (22 out of 37). However, band *5* also appeared in 7 samples of female gonangia and in 8 of the polyps. The frequency of the band combination *4* plus *6* or only *4* (*C* in Fig. 3) was considerably higher in extract of female gonangia than of polyps (16 out of 21). No male gonangia fell into group C.

The phosphatase profiles of *G. loveni* from the Gullmar Fjord fell into two groups, designated A and B, distinguished mainly by differences in the slowest bands *2–4* or *2–5* (*A* and *B* in Fig. 4). Furthermore, in the B-profiles, band *1*—at the starting zone—was markedly stronger and broader. In material from the Bergen area band *1* was missing and a slender band *5* replaced zone *5* (*B* in Fig. 4). Moreover, the additional band *9* was intensely coloured.

The species-specific phosphatase patterns of the three *Obelia* hydroids showed closely similarities in that slow, single bands or zones were present in addition to the broad, strongly coloured front (Figs. 5, 6). However, the electrophoretic separation was not as good as in the other genera, i.e. the bands in the *Obelia* hydroids were often replaced by diffuse zones. The migration rate of the strong front was fastest in *O. dichotoma* and slowest in *O. longissima* (Fig. 5). *O. dichotoma* displayed the largest individual variation, including also a slow variant (Fig. 6). The zymograms of *O. geniculata* and *O. longissima* were very similar. Sometimes only the different position of the fronts separated the two species (Fig. 6). In *O. geniculata* strong enzyme activity for the bands *2–3* was more common in extracts of gonangia compared with polyps. This

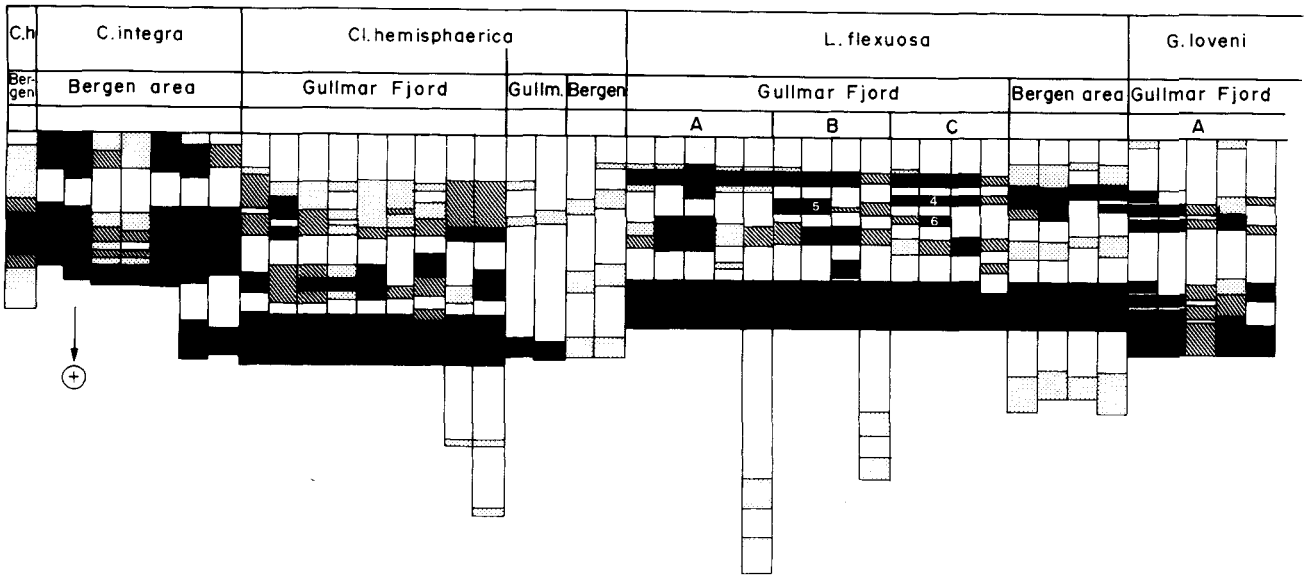


Fig. 1. Electrophoretic band patterns of acid phosphatases of hydroids. The migration rate of the broad, intensely coloured front is lowest in *Campanularia integra* and gradually higher in *Obelia longissima, O. geniculata, Laomedea flexuosa, Gonothyraea loveni, Clytia hemisphaerica* (the *johnstoni* and *gracilis* forms) and *O. dichotoma.* Note in particular the individual variation of *L. flexuosa.* The B-profiles show band *5*, commonly present in male gonangia and the C-profiles show the female bands *4* and *6*. In *G. loveni* from the Gullmar Fjord the A- and B-profiles are distinguished mainly by differences in the slowest bands. One additional, faster band is also present in *G. loveni* profiles from the Bergen area. *O. dichotoma* displays the strongest individual variation of the three *Obelia* species, including also a slow variant. In gonangia sample of *O. geniculata* from the Bergen area note the intensely coloured bands *2–3*. *C.h., Campanularia hincksii.*

was most obvious in the Bergen material (Fig. 1).

In the few samples of *C. hincksii* and the *gracilis* form of *Cl. hemisphaerica* (due to lack of material) only a few, mostly weak, bands were visible. The phosphatase patterns of *C. hincksii* showed a broad, slow band surrounded by weaker zones at the same electrophoretic position as the strong front of *C. integra* (Fig. 1). In the *gracilis* form, one strong band appeared at the same position as the front of the *johnstoni* form (Figs. 1 and 2).

3.2. *Esterases* (Fig. 7)

Complete esterase profiles were obtained only in a few samples of *L. flexuosa*, *O. geniculata*, *O. longissima* and *O. dichotoma.*The four species showed essentially the same basic patterns, composed of a number of consecutive single bands and an identical position of the front. The migration speed of a median band was almost similar in *L. flexuosa*, *O. geniculata* and *O. longissima*, but somewhat slower in *O. dichotoma* (Figs. 7, 8). The lowest number of bands appeared in *O. dichotoma* and the highest in *L. flexuosa*. In *L. flexuosa* sometimes one additional intermediate band 7 was visible (Fig. 8). This band was observed in 4 out of 7 samples of female gonangia, and one out of 9 of male gonangia. In the majority of runs only the slowest bands in the esterase zymograms appeared. Nevertheless, the additional species examined here, e.g. *C. hincksii*, *Cl. hemisphaerica* (the *johnstoni* form) and *G. loveni*, were readily distinguished when comparisons were made within the same gel.

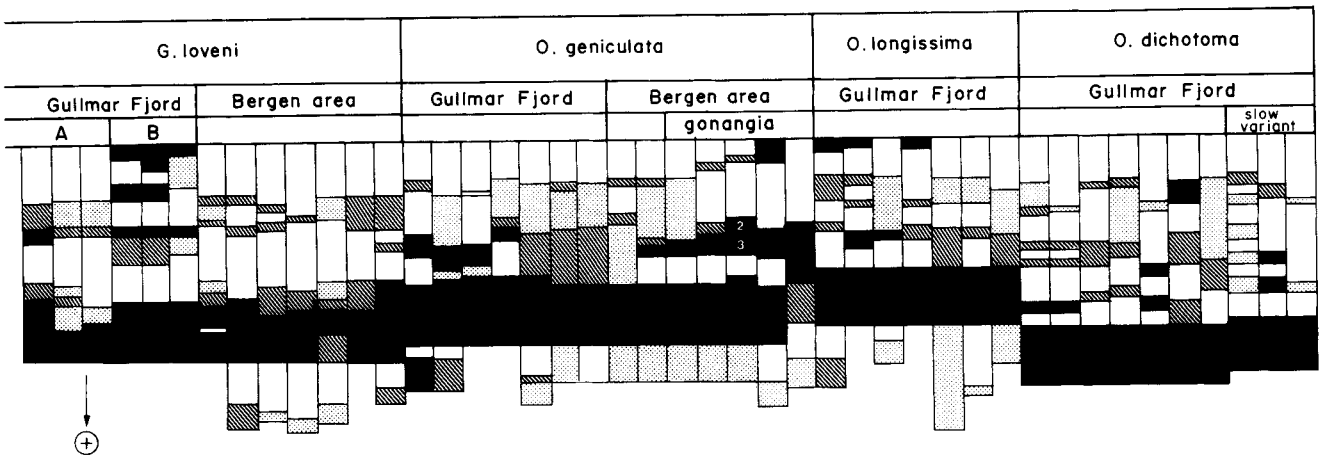
3.3. *Leucine aminopeptidases* (Fig. 9)

Only a minority of the samples analysed gave reproducible band patterns for leucine aminopeptidases. The common features for the successful runs were one broad, strongly coloured band or a complex of 2–3 bands of intermediate mobility and varying intensity. This complex was surrounded by single bands. Sometimes one band also appeared at the starting zone. The species could be recognized by the migration rate of their major complex of bands, which was highest in *L. flexuosa* and gradually declined in *C. integra*, *O. dichotoma*, *Cl. hemisphaerica* (the *johnstoni* form), *G. loveni*, *O. geniculata* and *O. longissima* (Figs. 9, 10). The largest number of bands, and the strongest intraspecific variation, were found in *L. flexuosa*. In 7 ot of 8 samples of female gonangia of *L. flexuosa* some of the bands in the complex were missing (Figs. 9, 10, ♀, *arrowheads*). The leucine aminopeptidase patterns of the *Obelia* species showed close resemblance, but the deviations from the other species were not large. The zymograms of *O. geniculata* and *O. longissima* were most similar and differed somewhat from that of *O. dichotoma.* In addition to a higher mobility of the complex of bands, a larger number of bands generally appeared in *O. dichotoma.*

4. Discussion

4.1. *The electrophoretic patterns*

The present electrophoretic study of campanulariid hydroids demonstrates that qualitative differences are manifest, at least at the level of species groups. The most suitable feature for species classification was the relative mobility of the fast, broad and strongly coloured front band of acid phosphatases (Fig. 1). In each species analysed for leucine aminopeptidase the electrophoretic mobility of the complex of bands obtained was also characteristic (Fig. 9). The esterase profiles were in most tests incomplete. however, the species examined were



recognized by the number and relative position of the slowest bands (Fig. 7).

Intraspecific variation regarding number and intensity of bands was present in all species analysed for the three enzyme systems. *L. flexuosa*, followed by *O. dichotoma*, showed the highest variability, when stained for acid phosphatases. Only minor dissimilarities were seen in the electrophoretic patterns between corresponding Bergen and Gullmar Fjord material (Fig. 1). Furthermore, different growth conditions within the two geographical areas (Table I) did not significantly affect the electrophoretic patterns. Moreover, sex seemed to have little effect on the isoenzyme patterns, except for *L. flexuosa*. The possibility cannot be excluded that some of the individual variations found may reflect differences in food intake.

4.2. *Taxonomical considerations*

4.2.1. *The genera* Campanularia, Clytia *and* Laomedea. Comparison of the phosphatase patterns of *C. integra* (and most likely of *C. hincksii*) with the patterns found in the other species examined (Figs. 1, 2) support the conventional assignment of *C. integra* (and probably *C. hincksii*) to the separate genus *Campanularia*. In *C. integra* the thickness of the periderm is highly variable and many irregularities occur in the shape of the gonothecae. Electrophoresis was performed on specimens with both thin-walled and grossly thickened hydrothecae and with smooth or spiral annulated gonothecae. The electrophoretic data suggest that the different morphological forms belong to the same species, supporting the notion of Broch (1910, 1918) and Millard (1966) that *C. calciculata* Hincks, 1853, distinguished by its thick-walled hydrothecae and smooth gonothecae, is a junior synonym of *C. integra*. However, Brock (1980) kept the two forms apart and treated them as separate species. No isoenzyme tests have been carried out with the compressed gonotheca (in cross-section) described in the *compressa* form (sensu Clark, 1876) of *C. integra*.

This relationship between the *gracilis* and the *johnstoni* forms of *Cl. hemisphaerica* was discussed in earlier papers (Östman 1979*a*, *b*). Due to the rarity of the *gracilis* form on the Swedish West coast, this hydroid has not been properly analysed electrophoretically although it would be of special interest in order to further clarify its taxonomic status. The enzyme activity analysed in *gracilis* samples was too low to yield distinct coloured isoenzyme patterns. The bands of the phosphatase zymograms of the *gracilis* form (Fig. 1), albeit being weakly stained, may indicate close relationship to the *johnstoni* form.

The phosphatase profile of *L. flexuosa* resembled the profiles of the *johnstoni* form of *Cl. hemisphaerica* (Figs. 2, 3). Moreover, *L. flexuosa* possesses a small b-rhabdoid nematocyst (Östman in prep.), which may be related to the large b-rhabdoid Type B nematocyst, occurring in *C. integra* and in the *johnstoni* and *gracilis* forms of *Cl. hemisphaerica* (Östman 1979*a*). These findings may reflect a relationship between the *flexuosa* hydroid and the *Campanularia* and *Clytia* species. However, due to the upright branching pattern of the *flexuosa* colony and its true diaphragm, this hydroid is here, in agreement with Cornelius (1981), placed in the genus *Laomedea* (see also Millard 1975).

4.2.2. *The genus* Gonothyraea. *G. loveni* differed from the other species examined regarding the acid phosphatase patterns (Figs. 1, 4). Thus, the electrophoretic data do not contradict the assignment of the *loveni* hydroid to the separate genus *Gonothyraea* as proposed by Cornelius (1975) and Millard (1975). Moreover, the nematocyst complement of *G. loveni* has been found to differ from other species of Campanulariidae (Östman in prep.).

4.2.3. *The genus* Obelia. In the Gullmar Fjord the two hydroids *O. longissima* and *O. dichotoma* differ in gross morphology and nematocyst structure (Östman in prep.). The typical branching pattern of *O. longissima*, in addition to the longitudinal striae from the incision between the low, rounded marginal teeth of the hydrothecae of *O. dichotoma* (cf. Kramp 1935) are here suggested to be species criteria. (The latter characteristic is not easily seen before the hydrothecae are stained with Chlorazol black). Furthermore, a b-rhabdoid nematocyst with notably long spines on its distal tube has been identified in *O. longissima*, and two different types of isorhizous nematocysts appeared in *O. dichotoma*. These nematocysts, which seemed to be species-specific, were present in both the polyps and medusae.

The electrophoretic patterns of acid phosphatase,

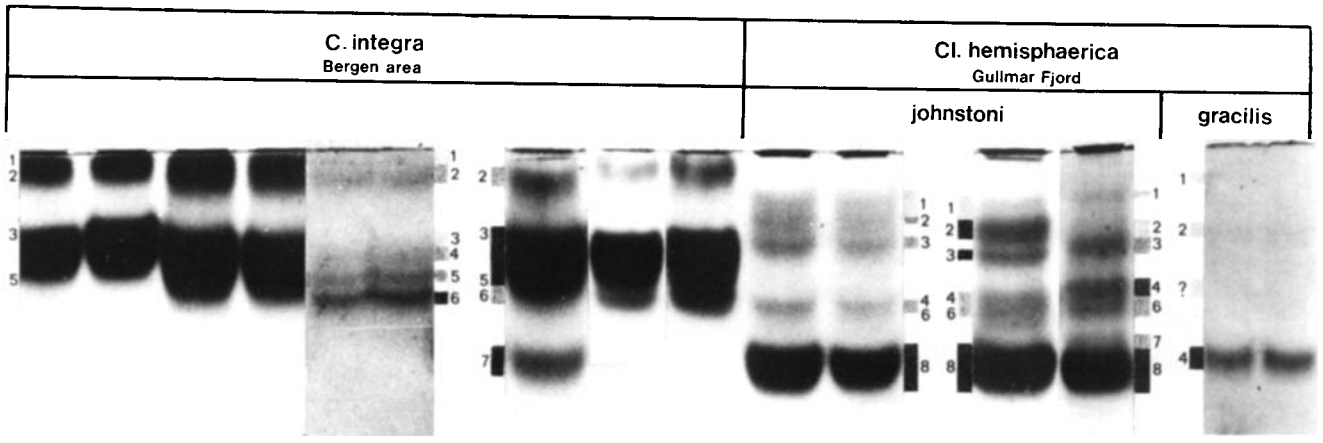


Fig. 2. Phosphatase zymograms of *Campanularia integra* and *Clytia hemisphaerica* (the *johnstoni* and *gracilis* forms). *C. integra* is recognized by the low mobility of the front bands *3–6* and by the broad, generally strongly stained single or divided bands *1* and *2* at or near the starting position. In the *gracilis* form the strong band *4* appears at the same position as the front band *8* of the *johnstoni* form. The other bands of the *gracilis* form are hardly visible.

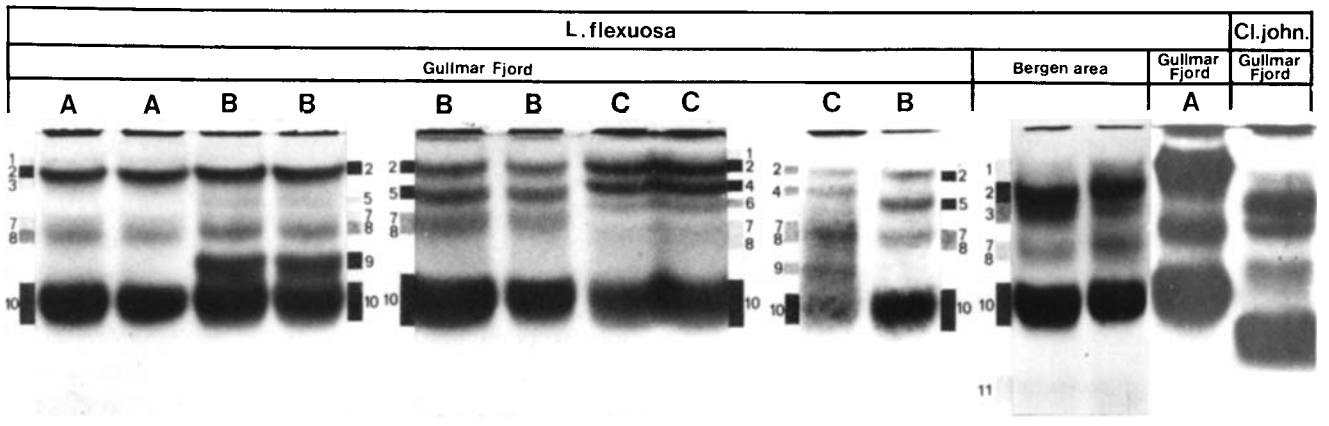


Fig. 3. Phosphatase patterns of *Laomedea flexuosa*. In B-profiles band *5*, characteristic of male gonangia, is visible. C-profiles display bands *4* and *6*, or only band *4* characteristic for female gonangia. Band *9*, which sometimes occurs, is seen in B- and C-profiles, and band *11* is present in samples from the Bergen area. The profile of *Clytia hemisphaerica* the *johnstoni* form is shown to enable a comparison of the position of the fronts.

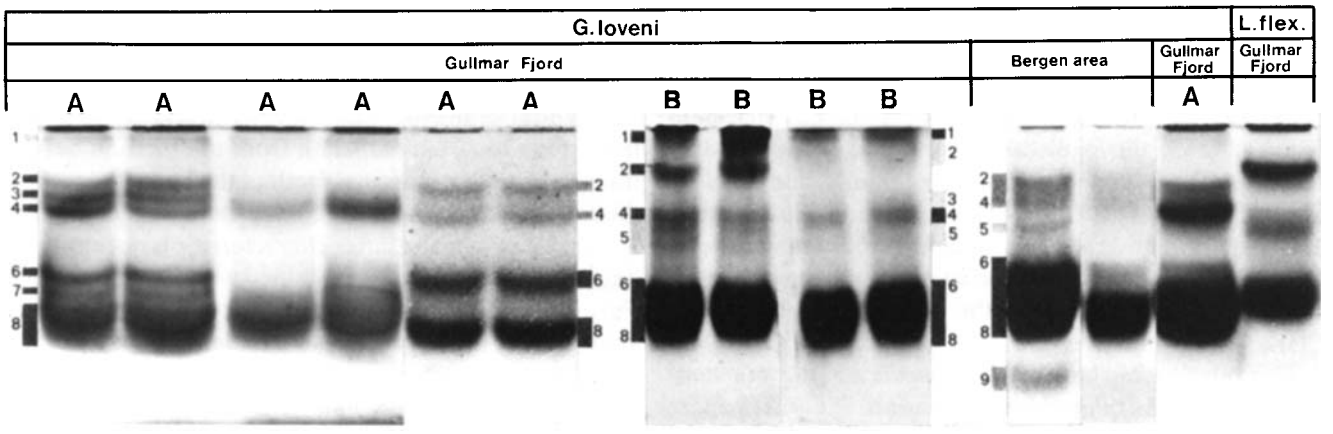


Fig. 4. Phosphatase patterns of *Gonothyraea loveni*. The B-profiles are separated from those of A mainly by means of the strong band *1* at the starting position and the appearance of band *5*. In zymograms from the Bergen area no band appears at the starting position, band *5* is slender and the additional band *9* is present. The last profile of *Laomedea flexuosa* is provided as a comparison.

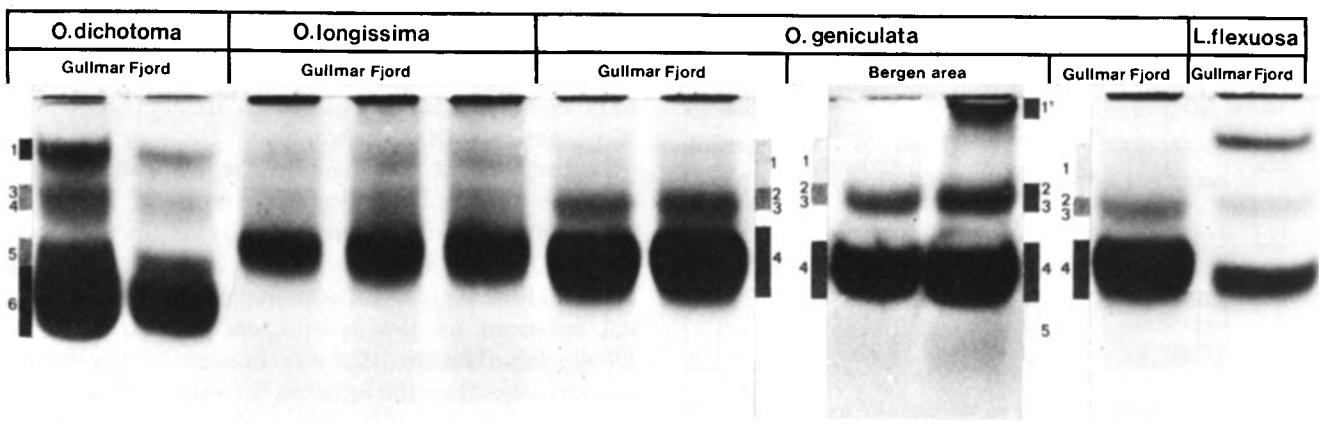


Fig. 5. Phosphatase patterns of *Obelia dichotoma, O. longissima* and *O. geniculata.* Band *1'* at the starting position of *O. geniculata* was only present in one profile of *O. geniculata* from the Bergen area. The zymogram of *Laomedea flexuosa* is provided as a comparison.

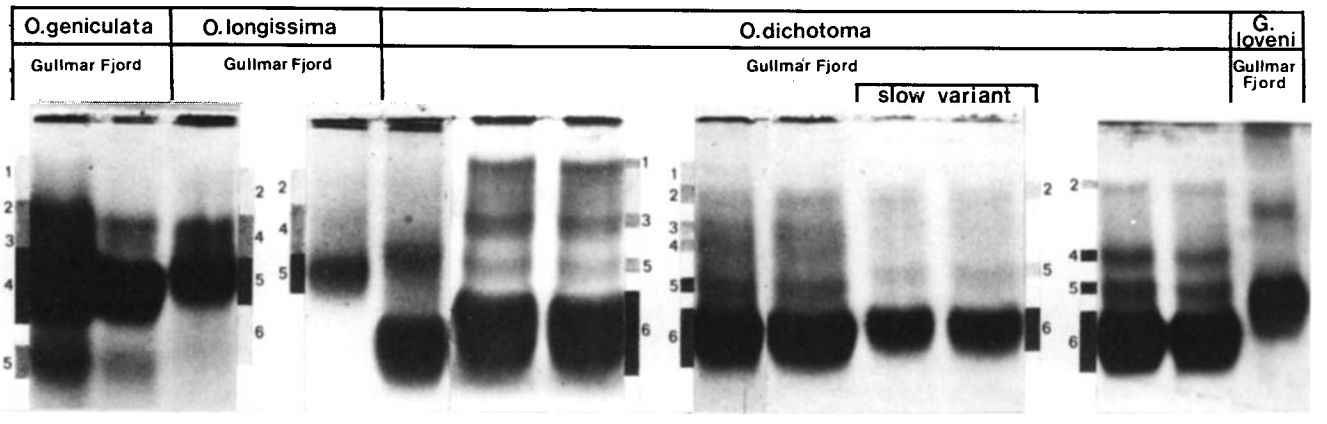


Fig. 6. Phosphatase patterns of *Obelia geniculata, O. longissima* and *O. dichotoma.* The zymograms of *O. geniculata* and *O. longissima* are very similar. Note in *O. dichotoma* the varying appearance of the bands *1, 2, 3, 4, 5* and the slow variant. The profile of *Gonothyraea loveni* is provided as a comparison.

esterase and leucine aminopeptidase of *O. dichotoma* and *O. longissima* displayed qualitative differences, which is a further confirmation of the distinctions between the two hydroids, and contradicts the theory that *O. longissima* is a junior synonym of *O. dichotoma* (Cornelius 1975). In conjunction with the morphological and ecological differences together with nematocyst studies (Östman, in prep.), the present results imply that *O. longissima* and *O. dichotoma* should be regarded as two relevant species (cf. Calder 1975). However, *O. longissima* and *O. plana* (M. Sars, 1835) may be synonymous (cf. Kubota 1981). The colony formation and the nematocysts of *O. plana* as described by Kubota (1976, 1981) seemed to be similar to those of *O. longissima.*

Obelia gelatinosa with *Obelia* medusae (sensu Hincks 1868), has been regarded as a synonym for *Laomedea gelatinosa* (Pallas, 1766) although the latter hydroid has sessile gonophores (Hartlaub 1897; Nutting 1915; Vervoort 1946). According to Hincks, *O. gelatinosa* is distinguished from *O. longissima* mainly by its compound stems and branches. Specimens with the same branching patterns as *O. longissima*, but with fascicled stems and branches—here suggested to be *O. gelatinosa* sensu Hincks—and typically unfascicled colonies of *O. longissima* deviate neither in their electrophoretic patterns nor in their cnidoms (Östman in prep.). Thus, *O. gelatinosa* with *Obelia* medusae sensu Hincks and *O. longissima* might be conspecific.

Cornelius (1975) put *O. plicata* Hincks, 1868 in synonymy with *O. dichotoma. O. plicata* was described by Hincks as having compound stems and branches, otherwise the hydroid was similar to *O. dichotoma.* Compari-

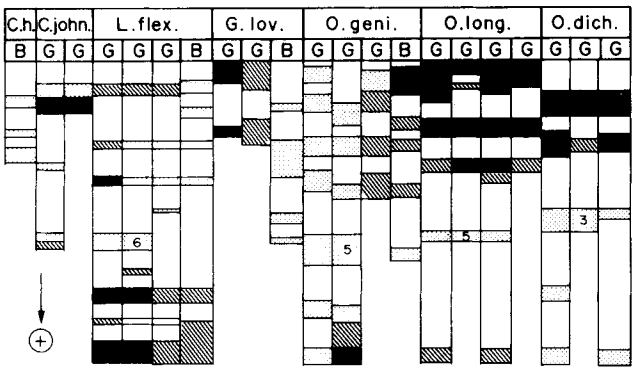


Fig. 7. Electrophoretic patterns of esterases of hydroids from the Gullmar Fjord (G) and the Bergen area (B). The electrophoretic position of the front is similar in the complete zymograms as are the positions of the median bands *6*, *5* and *5* of *Laomedea flexuosa*, *Obelia geniculata* and *O. longissima*, respectively. The migration rate of the median band *3* of *O. dichotoma*, however, is somewhat lower. *C.h.*, *Campanularia hincksii*.

sons of the electrophoretic patterns in specimens with the same branching patterns as *O. dichotoma*, but with fascicled stems and branches and typically unfascicled colonies of *O. dichotoma* confirm Cornelius' theory that *O. plicata* is conspecific with *O. dichotoma*.

In the present investigation the hydrothecae of *O. dichotoma* showed variation in the shape of the rim. The margin may be almost even or slightly undulating, like the typical *O. dichotoma* hydrothecae described by Nutting (1915). However, careful examination revealed longitudinal striations on the hydrothecae (cf. Kramp 1935). *Obelia dubia* Nutting, 1901 was characterized by longitudinal striations from the incisions between the low rounded marginal teeth. No differences in the electrophoretic patterns were observed to accompany dissimilarities in the shape of the hydrothecae. Therefore *O. dubia* is here considered as a synonym of *O. dichotoma*—in agreement with Millard (1966) and Cornelius (1975).

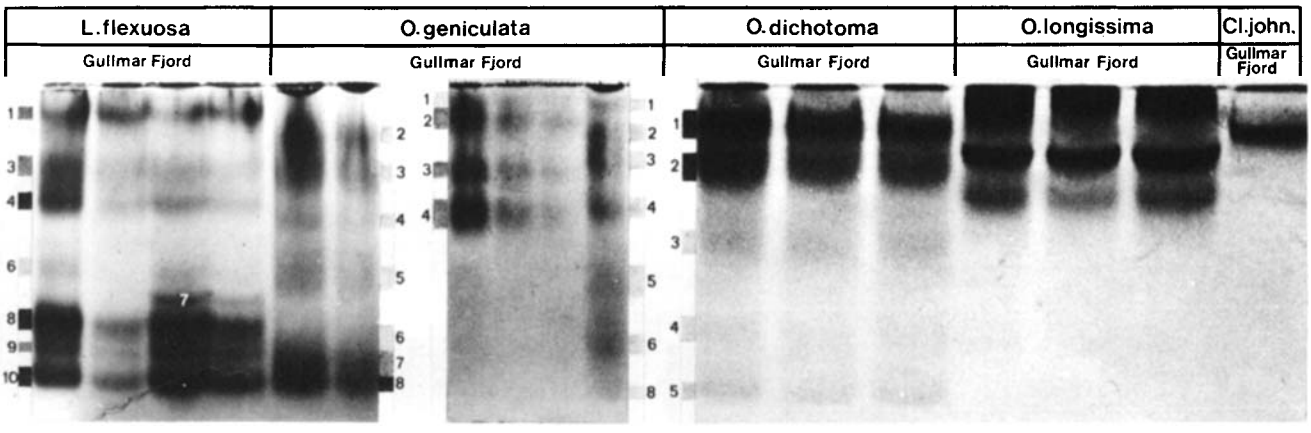


Fig. 8. Esterase zymograms of hydroids. The position of the fronts of the complete zymograms of *Laomedea flexuosa, Obelia geniculata* and *O. dichotoma* are similar, as are those of the median bands *6* and *5* of *E. flexuosa* and *O. geniculata*, respectively. In *O. dichotoma* the migration rate of the median band *3* is lower and only two slower bands are present. *O. longissima* and *Clytia hemisphaerica* f. *johnstoni* (*Cl. john.*) can be differentiated, although only few bands appear.

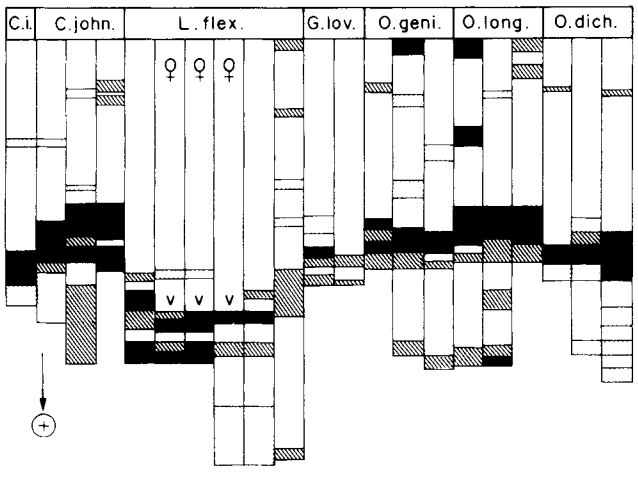


Fig. 9. Electrophoretic patterns of leucine aminopeptidases. The hydroids are from the Gullmar Fjord with the exception of *Campanularia integra* (*C.i.*), which was from the Bergen area. The migration rate of the intensely coloured complex of bands is highest in *Laomedea flexuosa* and lowest in *Obelia longissima*. In samples of female gonangia (♀) of *L. flexuosa* some bands are missing (*arrowheads*).

In *O. geniculata* the branching pattern and the thickness of periderm vary widely. However, the different morphological forms did not deviate in their isoenzyme patterns.

The close similarity of the phosphatase patterns of *O. geniculata*, *O. longissima* and *O. dichotoma* (Figs. 1, 5, 6) indicate a close relationship, and support the assignment of the three hydroids in the restricted genus *Obelia* (Cornelius 1975). The phosphatase patterns of *O. geniculata* and *O. longissima* in particular resembled each other. In some zymograms, only the faster mobility of the strongly stained front separated the two species (Figs. 5, 6). In *O. dichotoma* the number of bands, the migration speed of the strong front and the individual variation were higher than in the two other *Obelia* species (Fig. 6).

4.3. *General conclusions*

In conjunction with other criteria, polyacrylamide gel electrophoresis can serve as a valuable aid in hydroid taxonomy. Thus, for continued evaluation of taxonomic relations within Campanulariidae it seems desirable to include examination by electrophoresis of further species. For such a purpose, acid phosphatases seem to be the obvious enzyme system to study.

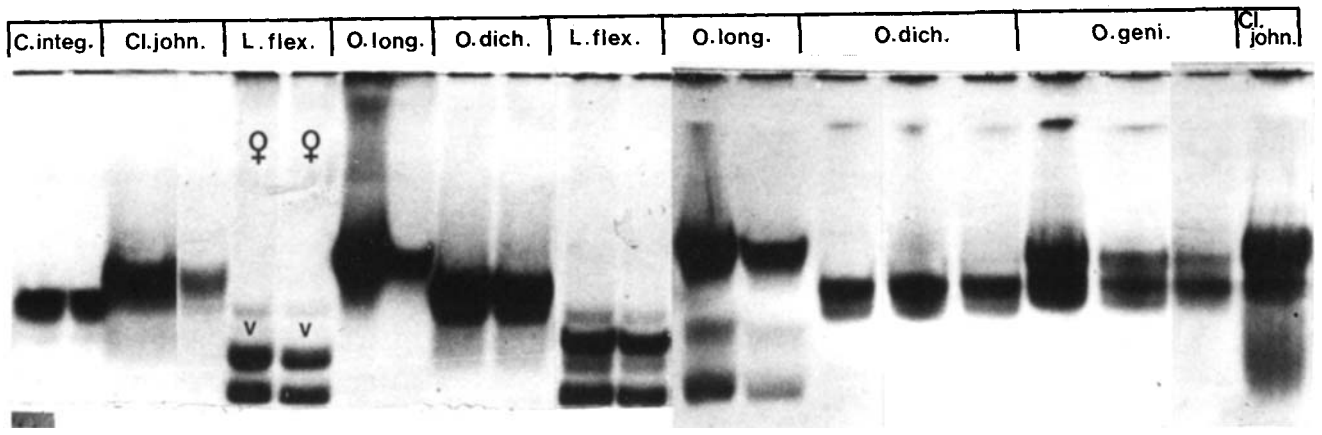


Fig. 10. Leucine aminopeptidase zymograms. The hydroids are from the Gullmar Fjord with the exception of *Campanularia integra*, which is from the Bergen area. The relative position of the complex of bands is shown. In samples of female gonangia (♀) of *Laomedea flexuosa* one of the bands in the complex is missing (*arrowheads*).

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