Journal of Nematology 19(4):431–440. 1987.

Two-dimensional Protein Patterns in *Labronema*, *Aporcelaimellus*, and *Eudorylaimus* (Nematoda: Dorylaimida)[1]

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Abstract: Two-dimensional polyacrylamide gel electrophoretic patterns of proteins for two isolates of *Labronema* from Indiana were nearly identical to the pattern for *L. vulvapapillatum* from Europe. The pattern for a nominal isolate of *L. pacificum* from Florida was very different from the patterns of nominal *L. pacificum* isolates from Hawaii and Fiji (which had patterns very similar to each other). Patterns for four other isolates (in *Eudorylaimus* and *Aporcelaimellus*) were different from the *Labronema* patterns and from each other, although some constellations of protein spots were shared among all the isolates. The study demonstrates the utility of 2-D PAGE for clarifying taxonomic problems that cannot be resolved using classical morphological data alone.

Key words: two-dimensional gel electrophoresis, 2-D PAGE protein patterns, *Labronema, Aporcelaimellus, Eudorylaimus,* Nematoda: Dorylaimida, systematics, Jaccard analysis, similarity coefficient.

Protein patterns obtained by two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) are proving useful to protozoologists, who must rely on nonmorphological criteria for taxonomic characterization and for study of systematic relationships (1,14). For nematode systematists, who traditionally rely on morphological characters for taxonomy and systematic inference, published investigations with 2-D PAGE patterns are limited to comparisons between two or three closely related species or subspecies at best, and several seem to convey the impression that such data are only marginally useful for nematode systematics (6).

Although systematic investigations in nematology must begin with morphological data, such data may be limited in their usefulness. Two-dimensional protein patterns can provide a new source of additional data at a molecular level. With such data evolutionary divergence among taxa and isolates of nominal taxa can be visualized and evaluated across a broad spectrum of different kinds of proteins, with the patterns probably reflecting degree of divergence over the entire genome.

We present 2-D PAGE protein patterns for six isolates of the dorylaimid genus *Labronema* Thorne from diverse areas, together with an analysis of the phenetic similarity of these six isolates based on 2-D PAGE protein data. For comparison, we also show 2-D protein patterns for isolates of *Eudorylaimus* Andrássy and *Aporcelaimellus* Heyns.

Materials and Methods

Isolates: Two isolates of *Labronema,* Lab-IN1 and Lab-IN2, were collected from woodland soil in Pulaski County and Howard County, Indiana, respectively, within the past 6 years. At the outset we assumed that both isolates were conspecific with *L. ferox* Thorne apud Ferris (4), based on size and general features, although we did not examine specimens under the compound microscope before comparing their protein patterns with other isolates. Lab-EUR is a subculture of a laboratory culture of the recently redescribed (12) *L. vulvapapillatum* (Meyl) sent to us in October 1982. This widely studied European culture (9,10,19) was isolated at least 10 years ago from greenhouse soil in Scotland (19), although the type locality of *L. vulvapapillatum* is the island of Ischia, Italy. Lab-FL, collected in 1966 from detritus in a trunk hole of a tree in Coral Gables, Florida, can be assigned to *Labronema pacificum* (Cobb)

Received for publication 12 January 1987.

[1] Journal paper 11132, Purdue University Agricultural Experiment Station, West Lafayette, IN 47907. Supported in part by NSF Grant No. BSR-8214460.

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We thank Mrs. Phyllis Lockard for technical assistance and Drs. S. R. Johnson, O. V. Holtzman, J. Rössner, and J. R. Williams for isolates.

based on morphological data. Lab-HI, collected in 1982 from soil in a wooded natural area of a nature center on the island of Hawaii, Hawaii, and Lab-FIJI, collected in 1983 from soil from a natural wooded area on Vita Levu, Fiji, can also be assigned to *L. pacificum* on the basis of the various published descriptions of that species, provided sufficient latitude is allowed for local variability and overlapping morphometric values (Ferris, unpubl.).

Data for four additional dorylaim isolates, selected for comparison of protein patterns because of their presumed close phylogenetic relationship to *Labronema*, are as follows: Apor-CAN, close to or conspecific with *Aporcelaimellus obscurus* Thorne & Swanger, was collected in 1980 from woodland soil in Vancouver, British Columbia, Canada. Eud-MAUR was collected in 1981 from soil around weeds in a sugar cane field in Magenta, Mauritius. Eud-HI was collected in 1983 from sugar cane soil in Kahaluu Valley, Oahu, Hawaii. Both Eud-MAUR and Eud-HI belong to the "granuliferous group" of *Eudorylaimus* Andràssy (18), although differences in tail length preclude their being conspecific. Eud-KAU was in detritus taken in 1982 from a trunk hole of a Koa tree (*Sapindus* sp.) at a high elevation along a mountain trail on Kauai island, Hawaii. This isolate has a body shape similar to that of *Eudorylaimus*, a long spear aperture more similar to *Aporcelaimellus* Heyns, and a short, rounded digitate tail not typical for either genus.

All of the isolates were maintained in culture in our laboratory. Voucher specimens for all isolates have been deposited in the Purdue Nematode Collection, and detailed morphological, cytological, and life history data, as well as taxonomic treatment, will be presented elsewhere.

Cultures: We received Lab-FL and Lab-EUR in culture and maintained them on 1% water agar using *Panagrellus redivivus* as prey. Cultures of the other eight isolates were started by methods similar to those previously reported (4) and were also maintained on *P. redivivus*.

2-D gels: Laboratory procedures were essentially as described elsewhere (7). For each isolate, ca. 200 adult specimens were picked from agar cultures. For the *Labronema* isolates, each sample consisted of approximately equal numbers of males and females. For the other isolates, which lacked males, only females were used. The specimens were cleaned by repeated rinsing in tap water, homogenized in 0.2 M sodium borate at pH 9 in an ice bath, and centrifuged. The supernatant liquid containing the proteins was dialyzed, stored over liquid nitrogen until labeled in vitro by reductive methylation with formaldehyde and sodium [^{3}H]borohydride (11), and subsequently stored at −80 C until gels were run. Two-dimensional polyacrylamide gel electrophoresis (2-D PAGE) was carried out essentially as described by O'Farrell (13). In 2-D PAGE, proteins are separated according to isoelectric point by isoelectric focusing in the first dimension, and according to molecular weight by sodium dodecyl sulfate (SDS) electrophoresis in the second dimension. Separation by these two unrelated parameters results in a nearly uniform distribution of protein spots across a 2-D gel and yields more information about protein mixtures than do protein separations in a single dimension. We ran proteins from each isolate in the same cell with proteins from other isolates to permit tracing of small variations in protein positions to unavoidable peculiarities in individual gel runs (7). Molecular weight standards were run, and the pH gradient was measured as described previously (7).

Following separation in two dimensions, labeled proteins were located by fluorography. Four to twelve patterns were obtained for each isolate, and the transparent autoradiographs were overlaid and compared directly. As is usual with 2-D gels, internal "landmark" spots were used to align autoradiographs for comparison, and proteins or polypeptides with identical electrophoretic properties were assumed to be identical (2). We divided each pattern into four sectors for analysis. Each reproducible protein spot in each sector was

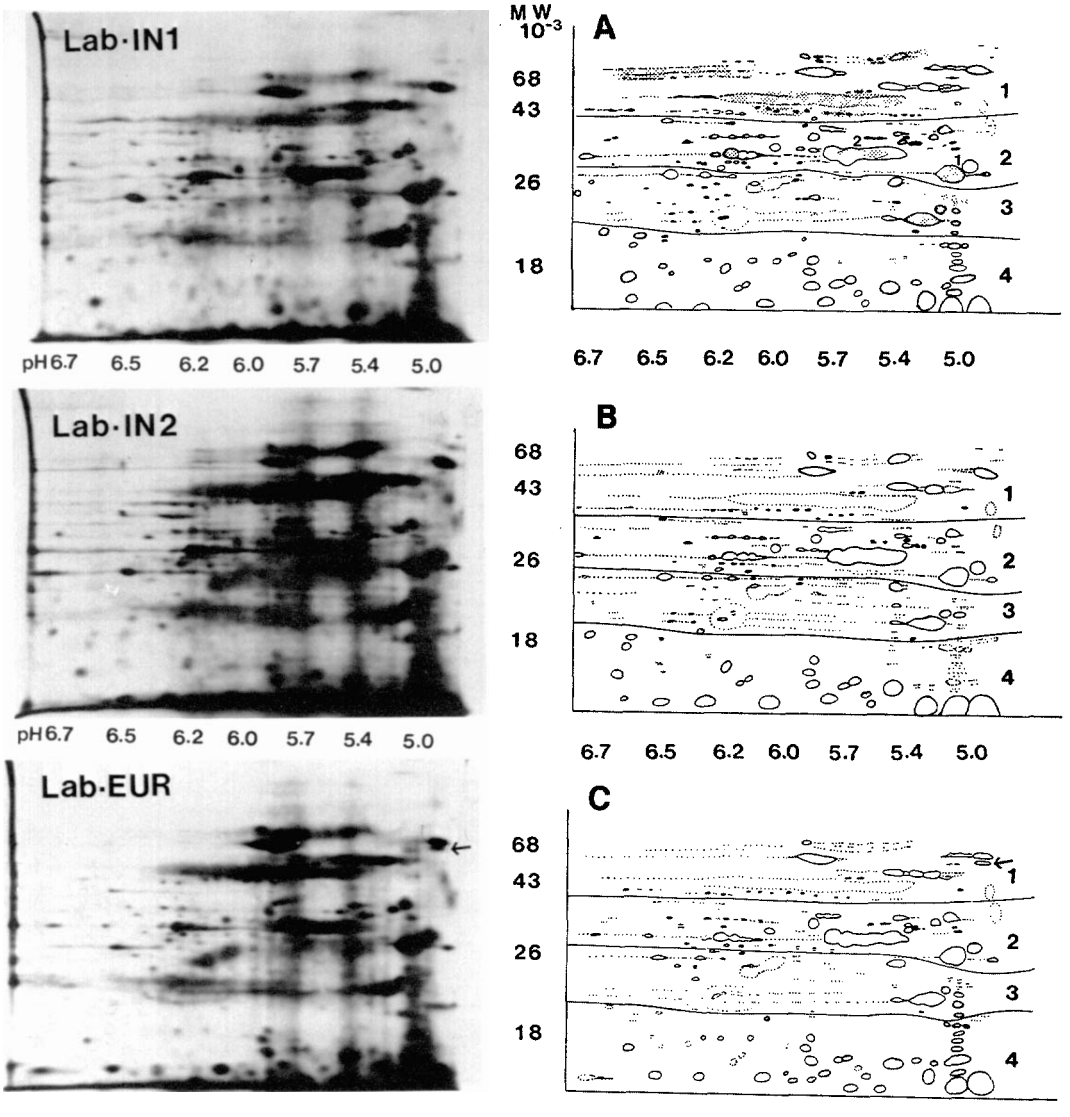

FIG. 1. Autoradiographs of 2-D PAGE protein patterns (left) and sketches (right) of typical patterns for *Labronema* isolates. A) Lab-IN1. B) Lab-IN2. C) Lab-EUR. Arrow in C indicates characteristic double protein spot in pattern for Lab-EUR. Stippled areas in A indicate symplesiomorphies between patterns of this group of isolates and patterns of all other isolates in study, as discussed in text.

numbered, and its presence or absence was determined for autoradiographs of all gels of all isolates. Only spots consistently showing good enough resolution to be easily recognized on multiple gels were scored (about 130 spots for each isolate). A spot consistently pale on some isolates and dark on others was scored as present in all isolates, with errors probably biased toward higher estimates of similarity rather than difference.

Jaccard analysis: The coefficient of Jaccard (3,15) to compute similarity coefficients (SC) was chosen for reasons described previously (8). The aim of the analysis was to estimate relative resemblance among the protein phenotypes of *Labronema* by calculating the percentage of

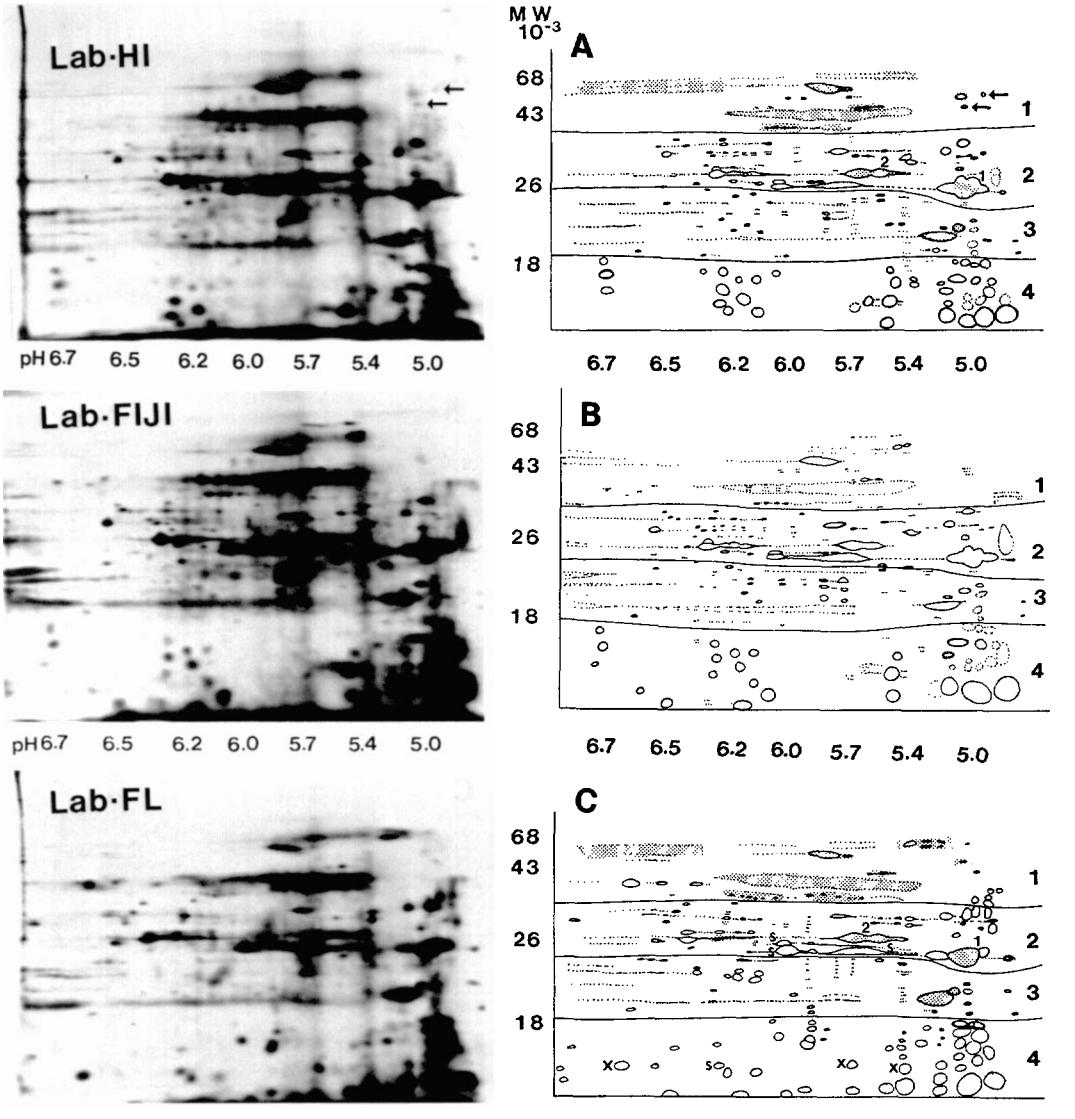

FIG. 2. Autoradiographs of 2-D PAGE protein patterns (left) and sketches (right) of typical patterns for *Labronema* isolates. A) Lab-HI. B) Lab-FIJI. C) Lab-FL. Arrows in A show small protein spots absent from the pattern in B. Stippled areas in A and C indicate symplesiomorphies as in Figure 1. In C, spots marked "s" are synapomorphic with spots in patterns for Lab-HI and Lab-FIJI (shown for these two isolates in Figure 6); and spots marked "x" indicate synapomorphies with other *Labronema* patterns (shown in Figure 5).

matching proteins. For two identical patterns, SC = 1.00.

RESULTS

Protein patterns for the two *Labronema* isolates from Indiana, Lab-IN1 and Lab-IN2 (Fig. 1A, B), were nearly identical to each other and to the pattern for the isolate from Europe, Lab-EUR (Fig. 1C). For the larger protein spots, the only consistent difference between Lab-EUR and the two Indiana isolates was that a single spot in sector 1 of the Indiana isolates always appeared to be double in Lab-EUR (indicated by arrow in Figure 1C).

The protein patterns for the two *Labronema* isolates from the Pacific, Lab-HI and Lab-FIJI, were very different from the pat-

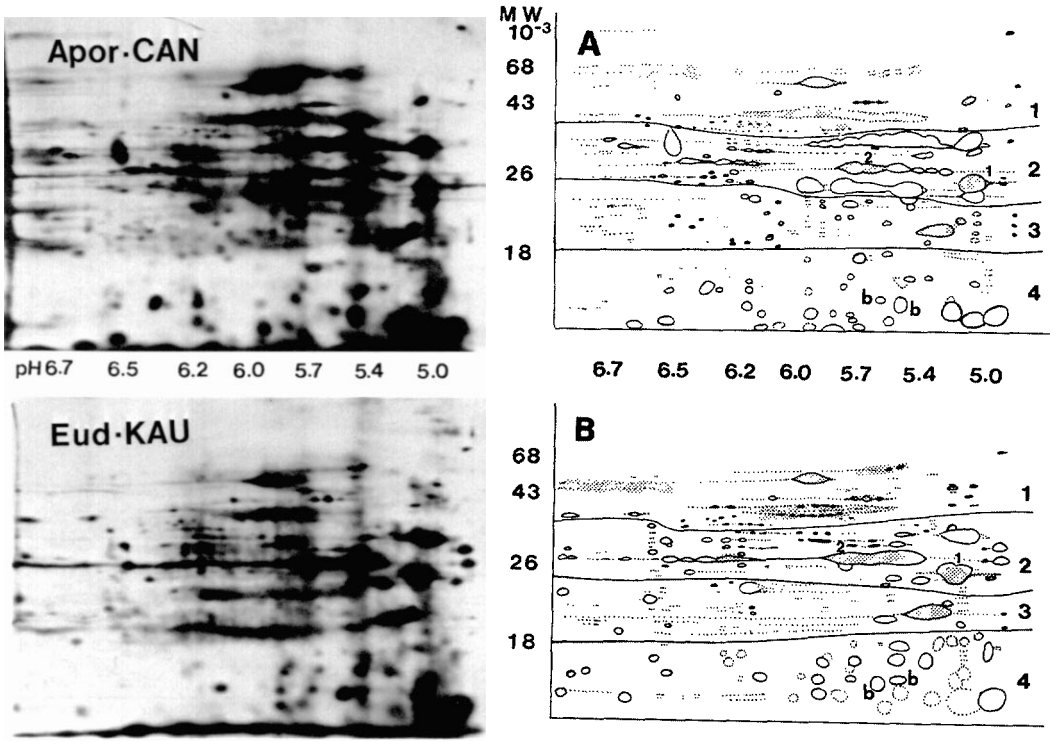

FIG. 3. Autoradiographs of 2-D PAGE protein patterns (left) and sketches (right) of typical patterns for isolates of *Aporcelaimellus* and *Eudorylaimus*. A) Apor-CAN. B) Eud-KAU. Stippled areas indicate symplesiomorphies as in Figure 1. Spots marked "b" indicate synapomorphies with Eud-MAUR and Eud-HI (shown for these isolates in Figure 7).

terns of Figure 1 but very similar to each other (Fig. 2A, B). A few differences between patterns of the two Pacific isolates were found for very small spots, e.g., the tiny spots in sector 1 indicated by arrows in Figure 2A. The pattern for the isolate from Florida, Lab-FL, differed markedly from all the other patterns (Fig. 2C).

The protein patterns for the *Aporcelaimellus* isolate, Apor-CAN, and the three isolates of *Eudorylaimus*—Eud-KAU, Eud-MAUR, and Eud-HI—differed from those of the *Labronema* isolates and from each other (Figs. 3, 4). The sketches accompanying the autoradiographs (Figs. 1–4) include those proteins scored after examination and comparison of all autoradiographs obtained for each isolate. The sketches represent typical patterns for the isolates and are not intended to be exact replicas of the accompanying photographs. In the autoradiographs of Figure 2, the pattern for Lab-FIJI appears to have extra spots near the bottom, not shared by Lab-HI. The separation of the lowest molecular weight spots in 2-D gels appears to be affected by a number of factors which vary between gel runs, and in other gels of Lab-HI we have found the extra row, although it is not shown in the typical pattern of the accompanying sketch (Fig. 2A).

Some of the protein and polypeptide spots shown in Figures 1–4 were shared by all of the patterns and are considered to be symplesiomorphies. Those larger spots found in all of the patterns are stippled in one sketch of each group of similar patterns (i.e., Figs. 1A, 2A, C) and in all sketches of Figures 3 and 4. In several instances patterns shared only a portion of a distinctive constellation of protein spots, e.g., constellation 1 in sector 2, in which a dense spot at the center of the constellation was shared by all the patterns, whereas pe-

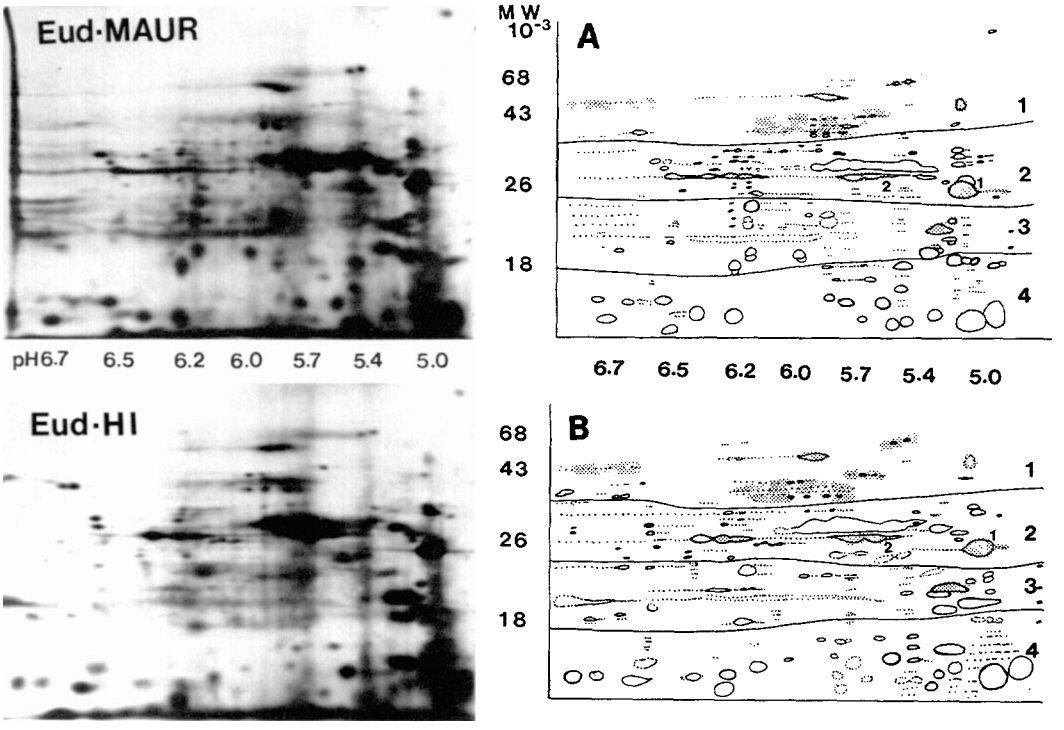

FIG. 4. Autoradiographs of 2-D PAGE protein patterns (left) and sketches (right) of typical patterns for *Eudorylaimus* isolates. A) Eud-MAUR. B) Eud-HI. Stippled areas indicate symplesiomorphies as in Figure 1.

ripheral spots varied among the patterns. A similar shared core of protein spots was present in constellation 2 of sector 2.

In addition to those protein and polypeptide spots shared by all the isolates in the study, certain other spots were shared only by sub-groups of the isolates. Many such spots were shared by the *Labronema* isolates from Indiana and Europe, and some of these special similarities (synapomorphies) are indicated by dark shading of the larger spots in the sketches of Figure 5. Some of the synapomorphic spots included spots peripheral to the symplesiomorphic spots discussed above, e.g., the additional proteins at the left in constellation 2 of sector 2. Many special similarities in proteins for these isolates occurred in sector 4, and of these, the three isolates shared three with Lab-FL (marked "a" on Figure 5 and "x" on sketch 2C).

The larger proteins and polypeptide spots shared only by Lab-HI and Lab-FIJI are indicated by shading on the sketches of Figure 6. Note the identical spots around the symplesiomorphic core of constellation 1, sector 2. A distinctive constellation of spots near the center of sector 2 (each spot marked "a" in Figure 6) was shared with Lab-FL (marked "s" on sketch 2C). In addition, the patterns for the two Pacific isolates shared a spot in sector 4 with Lab-FL (marked "a" in Figure 6 and "s" in Figure 2C). Many other synapomorphies between Lab-HI and Lab-FIJI were in sector 4 (Fig. 6).

The remaining four isolates shared very few synapomorphies with the *Labronema* isolates and with each other, except for Eud-MAUR and Eud-HI, which had a number of protein synapomorphies with each other (Fig. 7). Most of these were in sector 4, and for several of them the protein spot seemed consistently darker in one isolate than the other (indicated by different shading in Figure 7), although we scored the protein as "present" in both isolates. Two protein spots in sector 4 of Eud-MAUR and Eud-HI were also present in Apor-CAN and Eud-KAU (Fig. 7) but

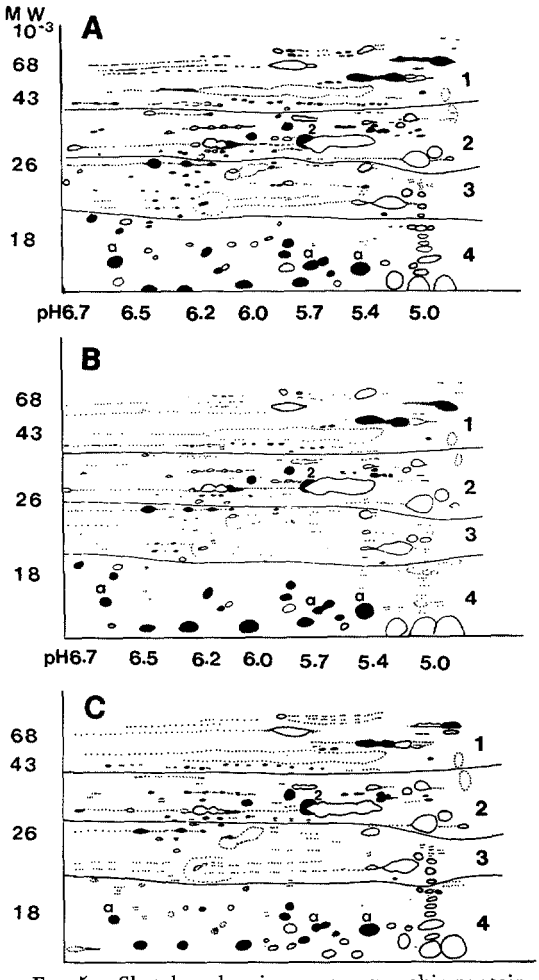

FIG. 5. Sketches showing synapomorphic protein spots of *Labronema* isolates as discussed in text. A) Lab-IN1. B) Lab-IN2. C) Lab-EUR. Dark-shaded spots indicate synapomorphies among this group of isolates. Shaded spots marked "a" indicate synapomorphies shared also with Lab-FL.

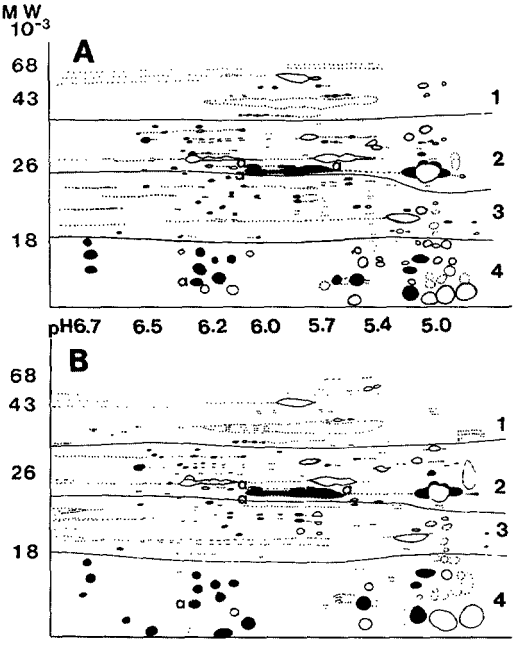

FIG. 6. Sketches showing synapomorphic protein spots of *Labronema* isolates as discussed in text. A) Lab-HI. B) Lab-FIJI. Dark-shaded spots indicate synapomorphies between these isolates. Shaded spots marked "a" indicate synapomorphies shared also with Lab-FL.

not in the patterns for the *Labronema* isolates (marked "b" in Figures 3, 7).

Because a substantial number of proteins were shared among the *Labronema* isolates, a Jaccard analysis was performed to obtain a quantitative estimate of phenetic similarity among the five isolates. The data for the other four isolates were not included because so many protein spots in each were not shared with any of the other isolates (autapomorphies) that an estimate of overall phenetic similarity seemed pointless. In the Jaccard analysis for the *Labronema* isolates (Table 1) Lab-EUR had an SC of 0.932 with the two Indiana isolates, which were identical to each other (SC = 1.000). Likewise, the two Pacific isolates had a high SC with each other (0.887). Lab-FL had a much lower pairwise SC with any of the others (0.333–0.389), with slightly lower pairwise SC with the Pacific isolates than with the others. The lowest SC were between the Pacific isolates and the isolates from Indiana and Europe (0.193–0.216).

DISCUSSION

At the outset of this study we assumed that Lab-IN1 and Lab-IN2 were isolates of *Labronema ferox* apud Ferris (4) because all three isolates were from central Indiana, they were about the same size, and they looked similar under the dissecting microscope. However, the protein patterns of Lab-IN1 and Lab-IN2 proved to be nearly identical to those of Lab-EUR. Close microscopic examination of specimens showed them not to be the same as *L. ferox* but to have the distinctive features of *L. vulva-*

papillatum, including the complex uterus, the ventral advulval papillae, and the elongate juvenile tail restricted to the first stage (12). Thus the morphological data as well as the protein patterns support the conclusion that the three isolates of the present study are all *L. vulvapapillatum*. Although we have fixed specimens of all stages of *L. ferox* apud Ferris (4), our living cultures were lost many years ago, so we have not been able to determine the protein pattern for *L. ferox*. We are attempting to isolate it again from the wooded area where we first found it, but we have been unsuccessful thus far.

Various descriptions of *Labronema pacificum* exist, based on specimens from Hawaii (type locality), Christmas Island, Jamaica, West Indies, and Mauritius. Specimens from all of these areas differ somewhat in size and in other features, and *L. pacificum* is a catch-all species name that could well accommodate all three of the isolates we tentatively assigned to it. The protein patterns, however, show conclusively that Lab-FL is different from the two isolates from the Pacific. Indeed, the protein patterns of Lab-HI and Lab-FIJI are very similar and probably represent isolates of the same species. Thorne (16) mentioned that specimens of *L. pacificum* from Hawaii (the type locality) had arcuate spicula rather than the L-shaped spicula originally drawn for the species (17). Of our isolates, both Lab-HI and Lab-FIJI have arcuate spicula, whereas Lab-FL has L-shaped spicula. Therefore, we conclude on the basis of all available data that Lab-HI and Lab-FIJI are isolates of *Labronema pacificum* and that Lab-FL belongs to a different, undescribed species.

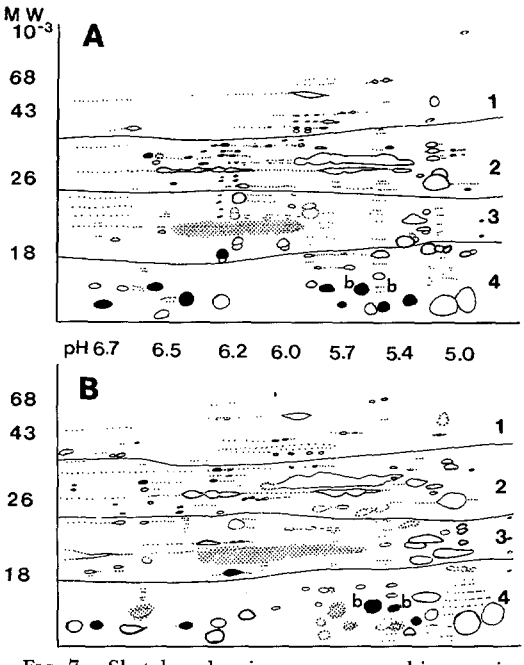

FIG. 7. Sketches showing synapomorphic protein spots of *Eudorylaimus* isolates as discussed in text. A) Eud-MAUR. B) Eud-HI. Shaded spots indicate synapomorphies between these isolates. Spots marked "b" indicate synapomorphies shared also with Apor-CAN and Eud-KAU.

This study demonstrates that the 2-D protein pattern is remarkably constant among geographically separated isolates of a species and apparently does not change rapidly owing to environmental differences. We have noted such constancy in 2-D protein patterns within species for a number of different taxonomic groups (5,7, unpubl.). It is likely that the widely separated geographical isolates of *L. vulvapapillatum* and *L. pacificum* have been separated for a long time (although other scenarios could be envisioned), and if so,

TABLE 1. Jaccard similarity matrix computed from 2-D PAGE data for six isolates of *Labronema*.

	Lab-IN1	Lab-IN2	Lab-EUR	Lab-FL	Lab-HI	Lab-FIJI
Lab-IN1	—					
Lab-IN2	1.000	—				
Lab-EUR	0.932	0.932	—			
Lab-FL	0.376	0.376	0.389	—		
Lab-HI	0.193	0.193	0.198	0.348	—	
Lab-FIJI	0.211	0.211	0.216	0.333	0.887	—

the conservatism in the 2-D gel patterns is surprising. Since separated populations might be expected to undergo random change over time at some level of organization, it is possible that more differences among the separated isolates might be found at the level of nucleic acids. In any event, it should be clear to any who fear the impact of increasing use of protein data on the stability of nematode taxonomy, that 2-D PAGE protein patterns can be a tool for affirming probable conspecificity of widely separated populations, as well as for challenging it.

The study also shows the utility of 2-D PAGE protein patterns for separating unlike entities that may seem to be quite similar based on morphological data. The confusion surrounding *L. pacificum* could not be resolved with data of classical morphology alone, but the striking differences in protein patterns preclude the assignment of Lab-FL to the same species as the two isolates from the Pacific. The capability for such resolution of long-term taxonomic problems should be welcomed by systematists, despite the fact that in some instances of very similar (but slightly different) patterns the taxonomic interpretation may not be as clear cut as for our nominal isolates of *L. pacificum*.

Perhaps the most interesting use of 2-D protein pattern data will be for phylogenetic inference. We emphasize that the similarities among the isolates of *Labronema* quantified by means of the Jaccard analysis are estimates of phenetic similarity only and are based on ancestral (plesiomorphic) as well as derived (synapomorphic) similarities. It is argued by some (for discussion see Ferris and Ferris [6]) that for molecular data, phylogenetic relationships can be retrieved from phenetic analysis, i.e., analysis that is based on overall (or raw) similarity. Others maintain that whatever the nature of the data, phylogenetic relationships can be determined only by analyses that utilize special similarity and group taxa on the basis of synapomorphies. Our next goal is to perform explicit phylogenetic analyses using the protein data for these 10 isolates.

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