Embryonic cell lineage evolution in nematodes of clade IV and V

Evolutie van embryonale celgenealogieën in nematoden van clade IV en V

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CHAPTER 1. INTRODUCTION

The development of nematodes is since long considered as an autonomous, mosaic development, where developmental potential is internally determined by differential segregation of the intrinsic determinants over the different blastomeres during cell cleavage (Boveri, 1899, 1910; zur Strassen, 1896; Stevens, 1909). In mosaic embryos, the differentiation potential of the early blastomeres do not depend upon interaction with neighbouring cells, and as a consequence such embryos have no regulative capacities to compensate for missing blastomeres. Most of the knowledge of the development of nematodes is mainly based on the model organism *Caenorhabditis elegans*. This nematode has a strict invariant cell lineage that generates a fixed number of cells with a fixed cell type (Sulston and Horvitz, 1977; Sulston et al., 1983). The reproducibility of the *C. elegans* cell lineage has the great advantage to study its development on a cellular basis and led to a vast knowledge in all fields of biology and medicine (Wood, 1988; Riddle et al., 1997; The *C. elegans* Research Community, 2005).

Initial experiments in *C. elegans*, where the development of isolated and cleavage arrested blastomeres was followed, indicated that at least the differentiation of the early blastomeres was determined by intrinsic determinants (Laufer et al., 1980; Cowan and McIntosh, 1985; Edgar and McGhee, 1986; Schierenberg, 1988). Also laser ablation experiments, where specific cells are killed by a laser microbeam, indicated that these cells are not replaced by others, suggesting a cell-autonomous development (Sulston et al., 1983). However, in further manipulation experiments, inducing signals between the early blastomeres have been identified that are necessary for the correct specification of these blastomeres (Priess and Thompson, 1987; Schierenberg, 1987; Schnabel, 1991; Wood, 1991; Bowerman, 1992; Goldstein, 1992, 1993, 1995).

Sulston et al. (1983) mentioned already, that in spite of the fixed relationship between the division pattern and cell fate, there seemed to be no obvious correlation between them, suggesting a complex patterning mechanism. More than twenty years later, these mechanisms are starting to be revealed as a complex mix of lineage-based and organ-based patterning mechanisms (Labouesse and Mango, 1999; Priess, 2005). In the early embryonic period, maternal genes

determine the embryonic axes and the identity and division pattern of the early blastomeres (lineage based mechanisms). Mutations in those genes affect the development of these blastomeres independent of the cell fate their descendants will form. In a second phase, during gastrulation, regions are formed consisting of cells from different lineages (organ-based mechanisms). In those regions, organ identity genes will determine the fate of the cells in each region. They concluded that *C. elegans* uses genetic tools, similar to vertebrates and *Drosophila* (eg, the use of the Wnt pathway in cell-cell signalling). However, it remains unclear how these evolutionary conserved mechanisms are used to form different body plans and more specifically, how these mechanisms evolved within the nematodes to give rise to the specific strategy of a complex, invariant cell lineage of *C. elegans*. Moreover, a recent large-scale EST project from 30 different nematode species uncovered a great genomic diversity in the phylum: 23% of the putative genes were unique to the species from which they were derived (Parkinson et al., 2004; Makedonka et al., 2005). Even between different *Caenorhabditis* species, the genetic distance in ribosomal and RNA polymerase genes is greater than across arthropod and vertebrate species (Kiontke et al., 2004). But it is still unclear how this genomic diversity reflects on the developmental diversity of nematodes. The study of the embryogenesis of more nematodes will teach us about the different strategies to form a nematode body plan and how these mechanisms are evolutionary linked to one another.

Recent research on other nematode species throughout the phylum uncovered much more diversity in developmental mechanisms. But most of this research is focussed on specific parts of development or specific developmental mechanisms, like the early development (reviewed in Schierenberg, 2006) or development of the gonad, vulva and male tail (reviewed in Sommer, 2005). Until recently, no detailed complete description of the embryonic development of any nematode beside *C. elegans* was available (Houthoofd et al., 2003). So, it remained unclear if these different mechanisms are part of a different mode of development or fit into the invariant "*C. elegans* like" mode of development. Bolker (1995) already warned for the extrapolation of developmental data of model organisms to other organisms. This data is often biased by the criteria these model organisms are often chosen. These include rapid development and fast generation time, developmental

canalisation and life history. Even historical accident and availability play a role. Rapid developing model organisms often share highly derived adaptations for this rapid development, such as prepatterning of the egg and maternal control. Secondly, model organisms are often chosen for their determined development with little variation and insensitivity for external cues, typically for a highly derived canalised mode of development. Is this cell lineage indeed a highly derived feature, typical for the fast and strictly determined development of *C. elegans* or is it a more general mode of nematode development?

To examine the extent of conservation of this mode of development in nematodes, we established the nearly complete embryonic cell lineages of three more distantly related nematodes. The marine free-living nematode *Pellioditis marina* TM02 belongs to the family Rhabditidae (clade V). The free-living nematode *Rhabditophanes* sp. KR 3021 is a sister species of the animal parasitic genus *Strongyloides* (Dorris et al., 2002). *Halicephalobus gingivalis* JB128 belongs to the family of the Panagrolaimidae. Both species belong to clade IV (Blaxter et al., 1998) (Fig. 1.1). Of these three species, we recorded their embryonic development from zygote to comma-stage embryo with a 4D microscope and followed each individual cell in time and space and established the complete division pattern or cell lineage by identifying all cell divisions. This gives us a unique data set of four embryonic cell lineages, which allows us a detailed comparison between these cell lineages on the level of each individual cell.

CHAPTER 2. MATERIALS AND METHODS

2.1. NEMATODE CULTURES

The free-living, marine nematode *Pellioditis marina* (Bastian, 1865) Andrássy, 1983 (Familia Rhabditidae, Ordo Rhabditida) was collected on rotting seaweed in the intertidal zone in Paje, Zanzibar by D. Verschelde. The strain TM02 is cultured on artificial sea agar plates (solution A: 23.9 g NaCl, 10.8 g $MgCl_2.6H_20$, 1.52 g $CaCl_2.2H_20$, 0.004 g $SrCl_2.6H_20$, 0.68 g KCl and 0.01 g KBr; dissolve to 856 ml; solution B: 40 g Na_2SO_4, 0.2 g $NaHCO_3$, 0.003 g NaF and 0.027g H_3BO_3 dissolve to 1000 ml. Mix solution A and solution B; buffer with TRIS-HCl to keep a neutral pH if necessary). This solution was used to make 1% agar plates (1/3 nutrient agar/agar); 1 ml cholesterol (5 mg/ml in EtOH) was added. *Escherichia coli* OP50 was used as a food source. Stock cultures were kept at 15°C; the maximum temperature of *P. marina* in culture is 25°C (Vancoppenolle et al., 1999). *P. marina* can also be maintained on typical *C. elegans* agar plates with *E. coli* OP50 as food source, but the culture declines over time and eventually dies out. All data presented here are derived from cultures on sea agar plates.

The soil nematodes, *Panagrobelus stammeri* PDL0024 Rühm, 1956*, P. redivivus* PS1163 (Linne, 1767) Goodey, 1945, *Halicephalobus gingivalis* JB128, Stefanski, 1954, *Panagrolaimus rigidus* AF36 (Schneider, 1866) Thorne, 1937, *Rhabditophanes* sp. KR3021 Fuchs, 1930 and *Cephalobus cubaensis* PS1197 Steiner, 1935 are cultured on 1% agar plates with *Escherichia coli* OP50 as food source. Culture and handling is as described by Brenner (1974).

2.2. 4D MICROSCOPY AND LINEAGE ANALYSIS

Early stage embryos from species where the embryos develop within the adult, are obtained by cutting gravid females in distilled water. Embryos from species that lay their eggs in the one-cell stage are collected by washing the eggs of the agar plate with distilled water. One-cell embryos are selected under a dissecting microscope, mounted on a 5% agar pad, covered with a coverslip and sealed with Vaseline (Sulston and Horvitz, 1977).

Three single embryos were recorded at 25°C using a 4D microscope (Schnabel et al., 1997; Houthoofd et al., 2003). Every 30 seconds, 25 focal planes were recorded through the embryo and stored on a laser videodisk. The recording starts at the 2-cell stage and continues until the body muscles start to contract; once muscle contraction starts, it is no longer possible to follow the cells between two time frames.

The lineage and 3D displays of each recording was reconstructed using the Simi Biocell software equipped with an automatic collision manager (version 3.5; 4.0, Simi Gmbh, D-85705 Unterschleissheim, Germany) (Schnabel et al., 1997). Since the recording ends at somatic body muscle contraction, it is not possible to always accurately identify the cell type of a given tissue. When discussing the nervous system, no distinction can always be made between neurons, sockets or sheaths; similarly, for cell types of the pharynx.

2.3. RELIABILITY

The lineage here presented is based on three recordings. A consensus lineage is formed, by which uncertainties in two complete recording were resolved by comparison with the third recording. As the recordings are restricted until body muscle contraction, some information of late development is not recorded. In our recordings the consensus lineage contains 571 cells and as such most cells later present in the L1, have already formed. Although there is some loss of lineage information, most cells have adopted their final fate and position. In *C. elegans*, 12 late divisions occur after muscle contraction: that means the formation of 12 extra cells over a total of 570 cells (or 2% of the number of cells) (Sulston et al., 1983).

2.4. NOMENCLATURE

The cells are named according to Sulston and Horvitz (1977), Deppe et al. (1978) and adapted by Sulston et al. (1983). Here we repeat shortly the nomenclature for better readability of the study. Founder cells formed in the first division rounds are given arbitrary names in capital letters according to Deppe et al. (1978). When a founder cell divides, each daughter is named by adding to the name of the mother cell a single low-case letter representing its position immediately after division relative to its sister cell. For divisions in anterior-

posterior direction the anterior and posterior daughter are indicated respectively with an ‘a’ and ‘p’, dorso-ventral divisions are indicated with ‘d’ and ‘v’, left-right divisions are indicated with ‘l’ and ‘r’. For example when founder cell E divides in anterior-posterior direction, the daughters are named Ea and Ep. When Ep divides in left-right direction its daughters are named Epl and Epr. A pair of cells may be designated by the use of internal parentheses, e.g., Ea(l/r)aa means Ealaa and Earaa. In the cell lineage tree the ‘a’, ‘d’ and ‘l’ daughters are represented by the left branches, and the ‘p’, ‘v’ and ‘r’ daughters by right branches. Long cell names are split in groups of three letters with spaces to improve the readability. For example, the cell ABalaaaaaaa, will be noted as ABala aaa aaa.

2.5. COMPARISON OF CELL LINEAGES

Considering the huge amount of data present in any given lineage, we have opted to sometimes include a comparison with the *C. elegans* embryonic cell lineage (Sulston et al., 1983) in the results section of each chapter, for clarity and to facilitate discussion.

When comparing the cell lineages two-by-two, we define two types of homology. Lineage homology between two species is calculated as the percentage of cells at time of muscle contraction of one species that have a homologues cell with the same lineage history in the other species. Fate homology between two species is calculated as the percentage of homologues cells at time of muscle contraction of one species that have the same cell fate in the other species.

2.6. PHYLOGENETIC ANALYSIS

The developmental data were mapped on a molecular phylogeny based on 18S rRNA sequences of the species treated in this study, all available in GenBank under accession numbers: *C. elegans* (**X03680**), *P. marina* (**AF083021**), *C. cubaensis* (**AF202161**), *Rhabditophanes* sp. (**AF202151**), *P. stammeri* (**AF202153**), *P. redivivus* (**AF083007**), *T. aceti* (**AF202165**), *H. gingivalis* (**AF202156**). The sequence of 18S rRNA of *P. rigidus* was sequenced for this study and is deposited in GenBank under accession number **Q285636**. *Plectus aquatilis* (**AF036602**) and *Mononchus truncatus* (**AY284762**) were used as

outgroup. The sequences were aligned using the program Clustal X under default settings (Thompson et al. 1997, version 1.64). Preliminary analysis demonstrated striking branch length differences within our dataset. In consequence, only model-based methods were implemented in our analyses as they incorporate better substitution bias (Swofford et al. 2001). The software Modeltest (Posada and Crandall 1998, version 3.06) was used to determine the best-fit maximum likelihood models; this was the general time reversible model (GTR+I+G). The parameters for base frequencies, substitution rate matrix, and shape and proportion of invariant sites were allowed to vary throughout the Bayesian analysis. The total number of generations in this analysis was set to 1 million, 300 times greater than the burn-in value. Four parallel chains (1 cold and 3 heated) were used. Trees were sampled every 100 generations. The burn-in value was set to 10,000 generations, which equated to the next 3,000 generations above the level at which the log likelihood reaches a stable value in a preliminary run. Majority-rule consensus trees were reconstructed from the fundamental trees. Phylogenies were estimated under maximum-likelihood criteria as implemented in PAUP (Swofford 1998, version 4b10) with bootstrap support from 100 replicates, and using Bayesian inference as implemented in MrBayes (Huelsenbeck and Ronquist 2001, version 3.0b4).

CHAPTER 3. EARLY DEVELOPMENT

3.1. FIRST DIVISIONS

The early development of *P. marina*, *Rhabditophanes* sp. and *H. gingivalis* start with a series of unequal, asynchronous cell divisions, during which a larger anterior somatic founder cell and a smaller posterior germ line precursor cell (P-cell) is formed (Fig. 3.1; Fig. 3.2, Fig. 3.3, Fig. 3.4). The zygote P_0 divides into an anterior somatic cell AB and a posterior germline cell P_1. This germline cell P_1 divides into an anterior somatic cell EMS and a posterior germline cell P_2. AB divides in a perpendicular direction. However, the dividing P_1 cell pushes one of the daughters of AB to the anterior side of the embryo; this cell will be assigned as ABa. The posterior daughter ABp moves posteriorly dorsally of EMS; this leads to the rhomboidal pattern (Fig. 3.2A). In *Rhabditophanes* sp. and *H. gingivalis,* these perpendicular division axes of AB and P_1 are clearly visible; resulting in a short transient T-shape that immediately converts to the rhomboid configuration (Fig. 3.4B). The position of EMS determines the ventral side of the embryo (Fig 3.4C). EMS divides into an anterior founder cell MS (mainly mesoderm) and a posterior cell E (endoderm) (Fig. 3.4D). The posterior daughter of the germline cell P_1 is called P_2 (Fig. 3.4C). In *P. marina* and *Rhabditophanes* sp., the polarity of the subsequent germline divisions reverses. P_2 divides in an anterior germline cell P_3 and a posterior somatic cell C (ecto- and mesoderm). Due to spatial constrictions of the eggshell, P_3 is pushed to the ventral side, and C is pushed to the dorsal side. Also P_3 gives rise to an anterior germline cell P_4 at the ventral side and a posterior somatic cell D (mesoderm) at the dorsal side of the embryo. This results in the typical configuration from ventral to dorsal: E-P_4-D-C (Fig. 3.2). In *H. gingivalis* there is only a partial reversal of cleavage in the germline. P_1 and P_2 arising from the posterior daughters (Fig. 3.4A,B). At the division of P_2, polarity of the germline reverses, so that P_3 arises from the anterior daughter lying ventral in the embryo comparable as in *Rhabditophanes* sp., *P. marina* and *C. elegans* (Fig. 3.4D). However at the division of P_3 the polarity reverses again so that P_4 arises from the anterior daughter and lies dorsally from its ventral sister D. As a result, the cells are oriented from ventral to dorsal: E-D-P_4-C (Fig. 3.4E).

The sequence of the first divisions is different in all studied species (Fig. 3.1; Table 3.1). The division sequence of *P. marina* is identical to in the *C. elegans* embryo except for the cleavage of P_3 that divides before C and the 8 AB cells (Fig. 3.1C). Consequently, the germ line precursor cell P_4 is already present in the 15-cell stage instead of the 24-cell stage in *C. elegans*. In one specimen, P_3 divides earlier, before MS and E, P_4 is already present in the 13-cell stage.

In *Rhabditophanes* sp. the founder cell AB divides little before its sister germ line cell P_1 (Fig. 3.1B, Table 3.2). After that, there is variation in the division sequence of the founder cells in different specimens. In one specimen, the two AB daughters divide before EMS and germline cell P_2, while in the other two specimens, 2AB divide after EMS and P_2. Also in the subsequent divisions there is variation between the three specimens: MS divides alternatively before, in between and after 4AB and E. The subsequent division sequence is constant: P_3 divides before C in all three specimens. The germline precursor P_4 arises at the same time, the 15-cell stage, in the three specimens.

In *H. gingivalis*, the founder cell AB divides at the same time as its sister germ line cell P_1 (Fig. 3.1D, Table 3.3). In the next division round, germline cell P_2 divides before the two AB daughters, ABa and ABp. EMS divides as the last granddaughter of the zygote. P_3 divides after MS and 4AB but before E; here the germline precursor P4 arises at the 14-cell stage. The division of E is delayed until after the division of the 8AB cells.

In *Rhabditophanes* sp., the eggshell does not tightly enclose the embryo. As a consequence, the early blastomeres are very motile during the early embryonic phase. This leads to variable transient configurations in the early embryo.

3.2. DEVELOPMENTAL TEMPO

The early development of *P. marina* is relatively slower in comparison with *C. elegans* (Fig. 3.5). The time between division of E and the division of E is 2.5 times longer than in the *C. elegans* embryo, while the period until muscle contraction is only 1.7 times slower than in *C. elegans* (Table 3.1). The development fastens after 200 minutes at a tempo similar to the developmental tempo in *C. elegans*

(Fig. 3.5). The developmental tempo of the embryonic development of *Rhabditophanes* sp. is comparable to that of *C. elegans* (Fig. 3.5). Also in *Rhabditophanes* sp. the early developmental period is slower than in *C. elegans* and speed up again after 200 minutes, the relative tempo is 1.2 until division of E and 1.1 until muscle contraction in comparison with *C. elegans* (Table 3.1). *H. gingivalis* has the slowest early developmental tempo, 2.9 times slower in comparison with *C. elegans* (Table 3.1). But also in *H. gingivalis* the developmental period speeds up, the period until muscle contraction is only 1.8 times slower than *C. elegans*.

In all species, the median cell cycle length of each AB generation increases during the course of embryonic development (Fig. 3.6). If one compares between the four species studied here, *C. elegans* and *Rhabditophanes* have an overall shorter median cell cycle length in AB than *H. gingivalis* and *P. marina*. Two different patterns can be distinguished. In *C. elegans* and *H. gingivalis,* the cell cycle length increases slowly at an equal rate. While the median cell cycle length of AB in *Rhabditophanes* sp. and *P. marina,* show a peak after which the tempo shortly speeds up again for one cell cycle. The position of the peak differs between those two species. In *P. marina,* there is a peak in cell cycle length at the 4AB generation, while in *Rhabditophanes* sp., the peak arises one cell cycle later in the 8AB generation.

After birth, each founder cell divides with characteristic periods (Fig. 3.7). The division rate correlates with the size and the time of origin of the founder cell: AB divides the fastest, D the slowest. E is an exception: it arises earlier than C but divides slower, the fourth generation of C divides earlier than the third generation of E. The length of the division rounds, that is the time between the first and the last division of each division round, also increases later in development. But the stages of the AB-lineage divide in a much shorter period than the corresponding stages of the other founder cells. For example, the 16-AB cell stage divides in the 6^{th} division round in a shorter period than the 16-MS stage, which divides in the 8^{th} division round. The length of the division rounds of each founder cell is much more correlated with the division round in the embryo than those of the founder cell. For example, in the 6^{th} division round, the 16-cell stage of AB and the 4-cell stage of MS divide in a comparative period of time.

3.3. BILATERAL SYMMETRY

In all studied species, the founder cells form bilateral symmetric groups in their first or second division round (Fig. 3.8). The anterior daughters of MS, C, D and P_4 lie in the left part of the embryo, while the posterior daughters lie in the right part. In AB and E, the left-right symmetry is established in the second division round.

In MS, E, D, and P_4 the bilateral symmetry is preserved for the next division rounds: bilateral symmetric cells are formed by equivalent lineages. In the E- and D-lineage the bilateral symmetry is preserved until after their last division round and is visible in the tissues they form. The intestine is completely formed by the bilateral symmetrical E-lineage and D forms bilateral symmetric muscle cells. In the MS-lineage the symmetry is retained until the 32-cell MS stage. The anterior daughters of MSa and MSp, MSaa and MSpa form a double inner row of eight cells, the posterior daughters, MSap and MSpp, which lie more lateral, form an double outer row. After the 7^{th} division round of MS the symmetry is lost.

In the third division round of C, a symmetry breakage in the body muscle precursors Cap and Cpp lineage is corrected to maintain the bilateral symmetry (Fig. 3.9). Cap and Cpp divide in a left-right direction. The outer daughters Capa and Cppp migrate anteriorly and lie before their sisters. Consequently, a secondary symmetry arises, where the descendants of Capa and Capp are symmetric with respectively Cppp and Cppa, hence the crossed lines in C in Fig. 3.8.

At the left–right division of the 2AB cell stage, a difference between the ABa and the ABp lineage occurs. The left daughters of the 2AB cells, ABal and ABpl, are skewed in the anterior direction, and their progeny lie on other positions as their lineal equivalents. In the ABa lineage the bilateral symmetry is completely lost while in the ABp lineage it is largely retained (Fig. 3.10). In *C. elegans*, the bilateral symmetry of the adult body plan is restored by non-symmetric lineages (Sulston et al., 1983). A total of 18 pairs of non-symmetrical sublineages give rise to symmetrical cells in the embryo (Table 3.4; Fig. 3.10) and later in the adult. Most of these secondary symmetries are already recognisable from the 200-cell stage in the embryo. These sublineages can be subdivided in two classes, equivalent left

and right lineages that give rise to symmetrical cells (eg. ABalp pp and ABpra aa) and lineages from the same side that give rise to symmetrical cells (eg. ABala pap and ABala ppp). Sixteen of those secondary symmetries are also scored in the *Rhabditophanes* sp. embryo, 14 in *H. gingivalis* and 13 in *P. marina* (Table 3.2; Fig. 3.11). There are possibly other secondary symmetries in these embryos that are present or not present in *C. elegans,* that are not recognised in these developmental stages. But since there is no post-embryonic data of these species, it is impossible to assign specific fate to cells within tissues (eg. subclasses of neurons) and recognise these symmetries as real symmetries that give rise to equivalent cells in the adult body plan.

3.4. GASTRULATION

In *P. marina,* gastrulation starts at 140 minutes, in the 24-cell stage with the inward migration of the two intestine precursors Ea and Ep from the ventral side, near the posterior end of the embryo (Movie 3.1). Next at 200-240 minutes P_4 and the MS cells migrate inwards. Between 250-300 minutes, D and the myogenous descendants of C sink inwards in the ventral posterior part of the embryo.

In *Rhabditophanes* sp., gastrulation starts at the 32-cell stage, with the stepwise ingression of the intestinal precursors derived by the E-lineage (Movie 3.2). At that time, the four granddaughters of E (Eal, Ear, Epl, Epr) lie in a square at the ventral side of the embryo. The two daughters of MS, MSa and MSp have divided obliquely, their posterior daughters MSap and MSpp, two mesodermal precursors, lie at the interior of the embryo, while the anterior daughters, MSaa and MSpa stay at the ventral side of the embryo in front of the four E cells. First, the posterior cells, Epl and Epr, ingress to the interior of the embryo, while the anterior daughters, Eal and Ear, stay at the ventral side of the embryo. The E cells divide again and the four anterior descendants of E stay at the ventral side. In a second phase at the 52-cell stage, these four anterior E-descendants ingress to the interior of the embryo which leaves a ventral cleft. Four MS descendants (MSaaa, MSaap, MSpaa, MSpap) at the anterior and the two germline precursors, P4a and P4p at the posterior migrate towards each other at the ventral side over the gastrulating intestinal precursors (102 cell stage). In a third fase, mesodermal precursors of the body muscles, derived from MS, D and 2 granddaughters of C,

Cap and Cpp, move inward from the ventro-lateral side into the ventral cleft. The P4 cells divide again, four germinal precursor cells lie in the ventral cleft and migrate after the mesodermal cells to the interior of the embryo.

In *H. gingivalis* gastrulation starts at the 29-44 cell stage, with the two intestinal precursors, Ea and Ep which lie ventrally, ingressing to the interior of the body (Movie 3.3). Ea gastrulates first at 180 min in between the mesodermal precursors MSap and MSpp just before the second division round of E. These MS cells gastrulate little after the two daughters of Ea. After the division of E, the two Ep daughters start to gastrulate. Right behind these cells, the big D founder cell divides in left-right orientation and its daughters Da and Dp gastrulate after the Ep daughters. Also the germinal precursor P_4 is divided and the two daughters migrate anteriorly in between the gastrulating D daughters. The MSaa and MSpa descendants divide anterior-posterior and migrate posteriorly over the gastrulating intestinal precursors. Next these cells migrate into the interior of the embryo. In the meanwhile, the mesodermal descendants of C, Cap and Cpp migrate from the dorsal side to the ventral side and lie on both sides of the two P_4 cells. In a final phase, these cells migrate together to the inside, as the AB descendants start to grow over the gastrulating cells.

CHAPTER 4. ORGANOGENESIS

4.1. LINEAGE AND FATE HOMOLOGY

4.1.1. *P. marina*

At muscle contraction, the embryonic consensus lineage of *P. marina* consists of 571 cells, 638 are formed of which 67 undergo programmed cell death (Fig. 4.1, Table 4.1; Table 4.2). Lineage homology shows an overall match of 95,5% with the *C. elegans* lineage (Fig. 4.2). Since reconstruction of the lineage ends at first somatic muscle contraction, we expect that the overall match might actually be higher because of some late divisions occurring after the end of the recording. Although in *C. elegans*, 12 late divisions occur after muscle contraction. That means the formation of 12 extra cells over a total of 570 cells (2%).

Fate homology, however, shows an overall match of 76,4% between *P. marina* and *C. elegans* (Fig. 4.2). A possible increase in overall match cannot be evaluated, since no predictions can be made about the fate of any cell formed after somatic muscle contraction.

4.1.2. *Rhabditophanes sp.*

At muscle contraction, the consensus lineage of *Rhabditophanes* sp. contains 606 terminal cells (Fig. 4.3, Table 4.1; Table 4.2). Of 552 of those cells (91 %) position and fate is determined (the position and fate of 36 cells and the division of 18 cells could not be resolved). The lineage similarity between *Rhabditophanes* sp. and *C. elegans* is 93.6%, which means that 517 of the 552 determined terminal cells have an equivalent terminal cell with the same lineage history in the *C. elegans* cell lineage (Fig. 4.2). However this is an underestimation since large part of the differences in lineage history of the cells is due to the mitoses that could not be resolved and the 29 late mitoses after muscle contraction in the *C. elegans* cell lineage that cannot be determined in *Rhabditophanes* sp. with 4D microscopy. The real lineage similarity is probably even higher between these species. When comparing the fate of each equivalent cell between *Rhabditophanes* sp. and *C.*

elegans, 454 of the 517 determined terminal cells (88%) in *Rhabditophanes* sp. have the same lineage history also have the same fate as in *C. elegans* (Fig. 4.2). Fate similarity with *P. marina* is only 82% (423 equivalent cells in *Rhabditophanes* sp. have equivalent fate in *P. marina*). Also the fate transformation is a possible underestimation, since the fate of 36 terminal cells and the cells generated by the 18 undetermined mitoses could not be determined.

4.1.3. *H. gingivalis*

At muscle contraction, the consensus lineage of *H. gingivalis* contains 597 terminal cells (Fig. 4.4, Table 4.1, Table 4.2). Of 536 of those cells (90%) position and fate is determined (the position and fate of 48 cells and the division of 13 cells could not be resolved). The lineage similarity with *C. elegans* and *P. marina* is 94%, 504 of the 536 determined cells have an equivalent terminal cell in *C. elegans* (Fig. 4.2). Fate similarity with *C. elegans* is 86%: 434 of these cells also have an equivalent cell fate. Fate similarity with *P. marina* is lower, 78% (only 395 of the equivalent cells in *H. gingivalis* have an equivalent fate in *P. marina*). Lineage similarity with *Rhabditophanes* sp. 92% (491 cells have equivalent lineage history in both species), while the fate similarity is 82% (437 of those cells have an equivalent cell fate). Like in *Rhabditophanes* sp., lineage and fate similarity with the other species is probably higher, since 48 cells in the *H. gingivalis* cell lineage have no determined fate and 13 cells have no resolved cell division.

4.2. INTESTINE

In all species studied so far, the intestinal cells are exclusively derived from the founder cell E, which does not give rise to any other tissue. The daughter cells Ea and Ep enter the body ventrally during gastrulation, except for *Rhabditophanes* sp. where gastrulation occurs in the 4E cell stage. The bilateral symmetry of the intestine in all the studied species is established in two different ways giving rise to two distinct patterns (Fig. 4.5, Fig. 4.6, Fig. 4.7).

In *H. gingivalis* (Fig. 4.5A, Fig. 4.6, Fig. 4.7 A-F, Movie 4.1A), the two daughters of E, Ea and Ep, divide in perpendicular division planes: Ea divides antero-posteriorly, Ep divides left-right (Fig. 4.6B, Fig. 4.7B). In the next division round,

the two daughters of Ea, Eaa and Eap divide asymmetrically in an antero-posterior plane (Fig. 4.6C, Fig. 4.7C). The two smaller posterior daughters Eaap and Eapp undergo programmed cell death. The larger anterior daughters migrate respectively to the left and right side of the intestinal primordium (Fig. 4.6D, Fig. 4.7D). In this way, a bilateral symmetric gut primordium of 6 cells arises. In the next division round all remaining cells divide antero-posteriorly to form a 12-cell primordium of 6 pairs of cells (Fig. 4.6E, Fig. 4.7E). The cells of the first, fifth and sixth pair divide once more to form three additional pairs. Each pair of cells will undergo morphogenesis to form a ring in the hollow tube. So, the *P. redivivus* intestine consists of 18 cells in two rows and nine rings (Fig. 4.6F, Fig. 4.7F)).

In *P. marina* and *Rhabditophanes* sp. (Fig. 4.7G-K, Movie 4.1B). The two daughter cells Ea and Ep divide left-right (Fig. 4.7H) and thereby establish the bilateral symmetry of the primordial intestine: Eal and Epl form the left row, Ear and Epr the right row. After the next two division rounds the intestinal tube is elongated and consists of two rows of eight cells (Fig. 4.7I,J). Ealp and Earp (respectively the light blue and light green cells in Fig. 4.7I) lie more ventrally according to the other intestinal precursors. Their anterior daughters Ea(l/r)pa migrate between Ea(l/r)aa and Ea(l/r)ap and its posterior daughter Ea(l/r)pp between Ep(l/r)aa and Ep(l/r)ap (Fig. 4.7J,K). The first pair of cells divides dorso-ventrally to form a ring of four cells, connecting the intestine with the pharynx (Fig. 4.7K). The last pair of cells Ep(l/r)pp divide once more to form a ninth row of intestinal cells that make contact with the rectum (Fig. 4.7K). In *Rhabditophanes* sp. it is the last but one pair of cells Ep(l/r)a that divides to form a 7th and 8th ring. In *P. marina,* the second to the fourth ring do not rotate 90° counter clockwise immediately after the division of the first pair of cells and before attachment to the pharyngo-intestinal valves as in *C. elegans* (Sulston et al., 1983; Leung et al., 1999).

Two species of the family Panagrolaimidae, *Panagrellus redivivus* and *Turbatrix aceti*, have an identical embryonic E-lineage as in *H. gingivalis,* in which 18 intestinal cells are formed and two precursors undergo cell death Sulston et al. (1983). However, the spatial organogenesis of the intestine of *P. redivivus* and *T. aceti* has not been described yet. To what extent are the differences in E-lineage translated in differences in spatial organization? Is this intestinal pattern common

for the family of the Panagrolaimidae or higher taxa or is there a high diversity in intestinal patterns in nematodes? How do these intestinal patterns relate to each other, which intestinal pattern is the ancestral one and which one the derived one? To address these questions, the spatial and temporal organogenesis of the embryonic intestine of *P. redivivus* and two other members of the Panagrolaimidae (*Panagrobelus stammeri* and *Panagrolaimus rigidus*) and one member of the Cephalobidae *Cephalobus cubaensis* was described and these results were plotted on a molecular phylogeny to infer the ancestral pattern.

4.2.1. Two distinct patterns lead to the same result

The bilateral symmetry of the intestine in all the studied species is established in two different ways giving rise to two distinct patterns, named after the species where the pattern was first described (Table 4.3). In *P. redivivus,* the intestinal morphogenesis is identical to the one of *H. gingivalis* as described earlier in Sulston et al. (1983) (Fig. 4.5A, Fig. 4.6, Fig. 4.7 A-F, Movie 4.1A). Eighteen intestinal cells are formed from the E blastomere and two precursors undergo programmed cell death. The three-dimensional cellular patterning during organogenesis of the intestine of *P. redivivus* and *H. gingivalis* will be called the *P. redivivus* pattern.

In *C. cubaensis*, *P. rigidus* and *P. stammeri* the embryonic intestine consists of 20 cells according to a pattern like in *Rhabditophanes, P. marina* and *C. elegans* (Sulston et al., 1983) and will be called the *C. elegans* pattern (Fig. 4.5B, Fig. 4.7G-K, Movie 4.1B).

Eventually in all the studied species, the embryonic intestine consists of 18-20 cells in two rows and nine rings. The most important difference between the two patterns is the establishment of the bilateral symmetry in the primordial intestine. In the *C. elegans* pattern this symmetry is established early in the 4E cell stage (Fig. 4.7H) by the left-right division of the two daughters of E, Ea and Ep. From this stage on Ea(l/r) and Ep(l/r) have an identical division pattern. In the *P. redivivus* pattern the bilateral symmetry is established later after the third division round of E when Eaaa migrates to the left side of the primordial intestine (Fig. 4.6D, Fig. 4.7D).

4.2.2. Differences in *P. redivivus* and *C. elegans* patterns are correlated with timing of gastrulation of mesodermal precursors

The difference in establishment of bilateral symmetry between the *P. redivivus* and *C. elegans* pattern is correlated with the timing of gastrulation of the two daughters of E in relation to the MS descendants MSap and MSpp (mainly mesoderm precursors). In the *C. elegans* pattern, Ea and Ep migrates inwards earlier than MSap and MSpp. Ea and Ep are therefore positioned more dorsally than the MS descendants during their division in left-right orientation (Fig. 4.7G). Similarly, during their subsequent division, the Ea daughters are positioned dorsally of the MSap and MSpp descendants in the *C. elegans* pattern (Fig. 4.7H). In contrast, in the *P. redivivus* pattern, Ea and Ep migrate inwards together with MSap and MSpp, so Ea is positioned between MSap and MSpp during its anterior-posterior division (Fig. 4.6A, Fig. 4.7A). The daughter cells Eaa and Eap are still positioned between the MSap and MSpp descendants during their gastrulation (Fig. 4.6B, Fig. 4.7B). After the subsequent division round, the daughters of Eaa and Eap lie more dorsally than the mesodermal precursors, derived from MSap and MSpp (Fig. 4.6C, Fig. 4.7C). The bilateral symmetry is re-established by the anterior daughters with the migration of Eaaa to the left side (Fig. 4.6D, Fig. 4.7D). The posterior daughters Ea(a/p)p undergo apoptosis.

4.2.3. Which pattern is the ancestral state within clade IV?

In order to reconstruct the evolutionary history of these two patterns, we calculated a molecular phylogeny of the studied species based on 18S rDNA sequences and plotted these two patterns on this phylogeny (Fig. 4.8). The results indicate that the *C. elegans* pattern is the ancestral state for clade IV and that the *P. redivivus* pattern has evolved from the *C. elegans* pattern. Secondly, the *P. redivivus* pattern has evolved within the family Panagrolaimidae. According to the 18S rDNA sequences, the *P. redivivus* pattern is a homoplastic trait within the clade (*P. redivivus*, *P. rigidus*, *H. gingivalis*, *T. aceti*), since the P. rigidus intestine has the *C. elegans* pattern. Based on the relationships in this phylogeny, there are two possible explanations. Either did the *P. redivivus* pattern arise twice independently in *P. redivivus* and the (*H. gingivalis*, *T. aceti*) clade or there was a

reversal in *P. rigidus* back to the ancestral *C. elegans* pattern. Another possibility would be that the homoplasy of this trait plotted this phylogeny is caused by homoplasy in the molecular characters of the 18S rDNA sequences that were used to reconstruct the phylogeny. This possibility can be tested in the future with different genes.

4.3. GONAD

In all studied species, the primordial gonad consists of germ line cells and somatic cells. Both have separate origins. The germ line cells are derived from the founder cell P_4. In *P. marina* and *H. gingivalis*, P_4 divides to form two germinal precursor cells. In *Rhabditophanes* sp., the primordial gonad consists of four germ line cells, due to an extra division round of P_4 (Table 4.2; Fig. 4.9). The somatic cells in all species studied here originate from identical lineages from MSa and MSp (MS(a/p) ppa ap) in the anterior part of the embryo and migrate posteriorly, where they attach to the germ line cells (Fig. 4.10). The primordial embryonic gonad is closely associated with the fifth intestinal ring (Fig. 4.11).

4.4. PHARYNX

In *P. marina*, there are 112 pharynx cells identified at the time of muscle contraction; 79 are generated by the founder cell AB and 33 by the founder cell MS (Table 4.2). In *Rhabditophanes* sp., 95 pharynx cells could be identified: 63 are formed by AB, 39 are formed by MS. In the primordial pharynx of the *H. gingivalis*, 91 pharynx cells could be identified: 60 cells are formed by AB, 31 cells are formed by MS.

The *P. marina* embryo has more pharynx cells than the other species. And only 60% of these cells have an equivalent pharynx cell in the other species (67 with *Rhabditophanes* sp., 64 with *H. gingivalis* and 65 with *C. elegans*). The other 40% of cells form mainly neuronal cells in the other species (23/45 in *Rhabditophanes sp,* 29/41 in *P. marina* and 28/47 in *C. elegans,* Table 4.6).

In all studied species, the pharynx potential is clearly restricted to specific sublineages (Fig. 4.12; Table 4.4). In the AB-lineage, pharynx cells are mainly formed by ABalp and ABara (64/79 cells in *P. marina*, 62/63 cells in

Rhabditophanes sp., 60/60 in *H. gingivalis*). In *P. marina,* 11 additional pharynx cells are formed by AB ala (1), AB plp (11) and AB prp (1). In the MS-lineage, the pharynx cells are mainly formed by the anterior daughters of MSa and MSp, MSaa and MSpa (29/33 cells in *P. marina*, 38/39 cells in *Rhabditophanes* sp., 31/31 in *H. gingivalis*). Since the MS cells gastrulate before the AB descendants, the MS-derived pharynx cells form primarily dorsal pharynx tissue, while the AB descendants form the ventral part of the pharynx. The initial bilateral symmetry of the early cell lineages is still visible in the primordial pharynx. MSaa forms pharynx cells on the left side of the pharynx; MSpa forms cells on the right side. In the AB-lineage the bilateral symmetry is not strict. ABara forms mainly cells on the right side; except for the AB ara apa lineage that forms cells on the left side together with the ABalp cells (Fig. 4.12).

At time of muscle contraction the pharyngeal primordium is visible as a distinct mass of cells in the anterior of the embryo. It is not possible to make a detailed 3D reconstruction of the pharynx and the different cell types, since the morphogenesis of the pharynx has not yet been completed. The pharynx elongates anteriorly and reaches the buccal cavity.

4.5. BODY MUSCLE

The 81 body muscle cells of the *P. marina* embryo have a mixed origin: 20 are generated by the blastomere D, 32 by C, 28 by MS and one by AB (Table 4.2; Fig. 4.14). These cells are arranged into four antero-posterior strips, two dorso-laterally and two ventro-laterally. The D blastomere forms exclusively body muscle cells. Bilateral symmetry is established after the first division: Da in the left part, Dp in the right part of the embryo. Two granddaughters of the founder cell C form each 16 body muscle cells: Cap at the left side, Cpp at the right side. The MS-derived muscle cells are bilaterally evenly distributed: 13 MSa cells and 1 MSp cell in the left muscle strips and 14 MSp cells in the right muscle strips. The founder cell AB forms 1 body muscle cell posteriorly in the right-ventral muscle strip.

In *Rhabditophanes* sp., the embryonic body muscle consists of 82 cells arranged in four antero-posterior bands, 2 dorso-lateral and two ventro-lateral. These cells arise from different sublineages: AB (1), MS (29), C (32) and D (20).

The configuration of the muscle cells at time of muscle contraction is identical to the one of *P. marina*, except for one MS cell, MSp pap pa, that divides once more to form an extra body muscle cell.

In *H. gingivalis,* 86 body muscle cell were identified. They arise from AB (1), MS (33), C (32) and D (20). However it was not possible to determine the spatial configuration in detail since not all muscle cells could be followed until time of body muscle contraction.

4.6. EPIDERMIS

The epidermis in *P. marina* has a mixed origin and comprises 132 cells: 115 AB cells, 3 MS cells and 14 C cells (Table 4.2). The epidermis has a polyclonal origin and comprises 86 cells, formed by the AB- (72) and C-lineage (14). In the *H. gingivalis* embryo, 90 epidermal cells of a polyclonal origin are identified. They arise from the AB- (74) and C-lineage (16).

The *P. marina* embryo has more epidermal cells than the other species (131 cells). And only 60% of these cells have an equivalent epidermal cell in the other species (82 with *Rhabditophanes* sp., 78 with *H. gingivalis* and 75 with *C. elegans*). The other 40% of cells form mainly neuronal cells in the other species (29/49 in *Rhabditophanes* sp*.,* 25/53 in *P. marina* and 32/56 in *C. elegans,* Table 4.7).

In the AB-lineage, epiderm cells are mainly formed in the posterior daughters of the ABa lineage by ABalp and ABarp (36/37 cells in *P. marina,* 30/32 cells in *Rhabditophanes* sp.; 28/28 cells in *H. gingivalis*) and in the anterior daughters of the ABp lineage by ABpla and ABpra (48/63 cells in *P. marina,* 37/40 in *Rhabditophanes* sp. 41/46 cells in *H. gingivalis*) (Table 4.4). ABpla and ABpra form bilaterally symmetric lateral fields while the epidermal ABarp descendants lie dorsally with bifurcated endings. In the C-lineage, epidermal cells are exclusively formed by the two anterior granddaughters Caa and Cpa.: 6 Caa-derived cells behind each other in the left dorsal field and 8 Cpa-derived cells behind each other in the right dorsal field.

All epidermal precursors arise in a dorsolateral region of the pre-morphogenetic embryo. The nuclei of the dorsal epidermal cells undergo contralateral migration at the onset of the morphogenesis (Movie 4.2). These dorsal cells elongate laterally and intercalate. The nuclei cross each other at the midline and migrate to the other side. These migrations are part of a larger morphogenetic movement of ectodermal cells towards the ventral side, called epiboly.

Some basic patterns in the organogenesis of the epidermis are identical to *C. elegans*, where ABpla- and ABpra-descendants form respectively left lateral and right lateral epidermis cells, while Caa-, Cpa- and ABarp-descendants form dorsal epidermis cells, the latter with the typical bifurcated endings. More variation is found in the epidermis cells that are specified later in the last division rounds.

4.7. NERVOUS SYSTEM

In *P. marina*, there are 194 neurons identified at the start of muscle contraction. 95% is formed by the AB-lineage (185 neurons), while MS forms 7 neurons and C only 2 (Table 4.2). 85% of the nerve cells formed in *P. marina* have an identical fate in *C. elegans*. 29 neurons are specific for *P. marina*. These cells in *C. elegans* undergo cell death (10), become pharynx (11), epidermis (2), divide once more (5) and one forms a pharyngo-intestinal valve cell. The 65 neuron cells, specific for *C. elegans*, form in *P. marina* pharynx cells (29), epidermis (33) and 3 undergo programmed cell death. Since reconstruction of the lineage ends at first body movement, we estimate that the number of nerve cells in *P. marina* is an underestimation of the actual number occurring in its embryogenesis.

In *Rhabditophanes* sp., there are 218 terminal neurons identified (Table 4.2). Those cells have mainly formed by the AB lineage (214/218 cells). The MS- and C-lineage both form two extra neurons. In AB, 76% of the neurons are formed by ABala (54), ABalp (29), ABplp (36) and ABprp (44) (Table 4.4). 67-79% of those 218 neuronal cells in *Rhabditophanes* sp. have an equivalent neuron in the other species (Table 4.8). Cells that form neurons that are specific for *Rhabditophanes,* become mainly cell deaths (49), epidermis (38) and pharynx (25).

In the *H. gingivalis* embryo, there are 200 terminal neurons identified (Table 4.2). In AB, 73% of the neurons are formed by ABala (37), ABalp (28), ABplp (40) and ABprp (41) (Table 4.4). One MS cell, MSa aaa aal, forms one extra neuron. 66%-82% of those 200 neuronal cells in *H. gingivalis* have an equivalent neuron in the other species (Table 4.8). Cells that form neurons that are specific for *Rhabditophanes* sp.*,* become mainly cell deaths (28), epidermis (32) and pharynx (32).

4.8. PROGRAMMED CELL DEATH

In *P. marina*, 11% (67/638; *C. elegans* 16%, 106/656) of the cells undergo apoptosis (Table 4.2, Table 4.5). All cell deaths occur in the AB- (58) and the MS-lineage (9) in the anterior half of the embryo and none in the C-lineage, unlike in *C. elegans*. 95% of the cell deaths in *P. marina* occur also in *C. elegans*, including the 14 prominent cell deaths in the 9^{th} generation of AB and the one early cell death in the 4^{th} generation of MS. There are 2 cell deaths in *P. marina*, which become neurons in *C. elegans* and one cell that undergoes apoptosis in *P. marina* divides once more in *C. elegans*. The difference between *P. marina* and *C. elegans* lies in the cell deaths after the last division round. Since reconstruction of the lineage ends at first body movement, we estimate that the number of cell deaths in *P. marina* is an underestimation of the actual number occurring in its embryogenesis.

Only 21 programmed cell deaths could be identified in the *Rhabditophanes* sp. embryo (Table 4.2, Table 4.5). All these cells have an equivalent cell death in *C. elegans* (Table 4.9) and 15 of those cells are early cell deaths after the ninth division round. The 71 cells that undergo cell death in *C. elegans*, but not in *Rhabditophanes* sp., become neurons (35), pharynx (10) and epidermis (2). The position and fate of 15 cells, which have a cell death in *C. elegans*, could not be resolved or the division of their mother cell (8 cells) could not be determined in the *Rhabditophanes* sp. recordings. It is possible that these cells also undergo cell death, but this could not be confirmed in the recordings.

Also in the *H. gingivalis* embryo, only 24 programmed cell deaths could be identified (Table 4.2, Table 4.5). Twenty of those cells have an equivalent cell death in *C. elegans* (Table 4.9). Four cell deaths are specific for *H. gingivalis*, one

in AB (ABala ppp aaa), one in MS (MSa apa pa) and two in the E-lineage (Ea(a/p)p); the equivalent cells in *C. elegans* respectively form a neuron, the MS-cell divides with the posterior daughter that undergoes cell death, and the two E-cells divide to form each two intestinal cells. The 72 cells that undergo cell death in *C. elegans*, but not in *H. gingivalis*, become neurons (24), pharynx (11), epidermis (6) and body muscle (2) (Table 4.9). The position and fate of 23 cells, which have a cell death in *C. elegans*, could not be resolved or the division of their mother cell (6 cells) could not be determined in the *H. gingivalis* recordings. It is possible that these cells also undergo cell death, but this could not be confirmed in the recordings.

CHAPTER 5. DISCUSSION

5.1. EARLY DEVELOPMENT

5.1.1. Reversal of polarity in the germline

In *P. marina* and *Rhabditophanes* sp.*,* the polarity of the germline divisions changes: P_1 and P_2 arise from the posterior daughters, and P_3 and P_4 arise from the anterior daughters. This phenomenon has also been described and extensively studied in the model organism *C. elegans*. Two different mechanisms are involved in generating the asymmetry and polarity of the germline divisions. The polarity and the asymmetry of the division of P_0 and P_1 is determined intrinsically by asymmetrically localized proteins, known as the *par* genes (Kemphues et al., 1988; Rose and Kemphues, 1998; Kemphues and Strome, 1997; Gönzy and Rose, 2005) (Fig 5.1 A,B). In contrast, the polarity of the divisions of P_2 and P_3 are determined by one signalling gene: *mes-1,* which encodes for a cortical protein, localized between gut precursors (EMS, E) and the germline (P_2, P_3) (Strome et al., 1995; Berkowitz and Strome, 2000) (Fig. 5.1 C,D). MES-1 acts in concert with the SRC-1 protein in the adjacent EMS cell to produce a bidirectional signal that controls the fate and division orientation in both EMS and P_2 (Bei et al., 2002) (Fig. 5.1C). After this division MES-1 congregates in the cortex of P_3 at the contact side with E, regulating the correct division axis and the correct segregation of the P-granules or germ granules into P_4 (Berkowitz and Strome, 2000) (Fig. 5.1D). In *H. gingivalis*, P_1 and P_2 arise from posterior daughters as in *P. marina, Rhabditophanes* sp. and *C. elegans* (Fig. 5.1 F,G). Also, the polarity of P_2 division reverses, resulting in an anterior germ cell P_3 and posterior cell C, as in *P. marina, Rhabditophanes* sp. and *C. elegans* (Fig. 5.1 I). But in contrast with these species, the polarity of the subsequent germline division of P_3 reverses again, resulting in a posterior germline precursor P_4 (Fig. 5.1 J). The resulting configuration of the posterior cells is: E-D-P4-C. After the last division, contact between E and the germline is lost. Other variations in the polarity of the germline divisions are found in other species. The cephalobid *Acrobeloides nanus* lacks a reversal of polarity; all the P-cells arise from the posterior daughters, resulting in a configuration E-C-

D-P_4 (Skiba and Schierenberg, 1992) (Fig. 5.1 K-O). In two *Rhabditis* species*, R. dolichura* and *R. belari,* the reversal of polarity is only expressed in the germline division of P_3, resulting in a configuration E-C-P_4-D (Laugsch and Schierenberg, 2004). Berkowitz and Strome (2000) suggested that in *A. nanus*, which lacks the reversal of polarity, either MES-1 must be lacking or that MES-1 must be used in another fashion. The other variations found in *H. gingivalis* and the *Rhabditis* species with partial reversals of polarities, favors for the second possibility, that a MES-1 like mechanism is used in several ways in those species. In *H. gingivalis,* the division of P_2 occurs as in *C. elegans*, with the future germline pole to the side of EMS. If in *H. gingivalis mes-1* determines germline polarity, variation in the mechanism should only occur in the last germline division of P_3. In *A. nanus*, *R. belari* and *R. dolichura*, the germline is immediately uncoupled from the endodermal line after the division of P_2. If also in these species *mes-1* plays a role germline polarity, then the dual function of MES-1, regulating division polarity and fate of EMS and P_2, has to be uncoupled, since the polarity of both spindles now occur to opposite directions, the spindles are skewed to opposite membranes. Alternatively, different mechanism can be used to polarise the germline instead of *mes-1*. For example, a PAR-like mechanism may continue in *A. nanus* specifying the P_3 and P_4 cell at posterior end like in the division of P_0 and P_1 in *C. elegans*).

Despite the loss of contact between germline and endodermal line in all these species with partial or no reversal of polarity, this contact is restored before the onset of gastrulation through cell migrations. In *H. gingivalis,* the two germline precursors migrate in between the two daughters of D, after which they gastrulate together. In the 6-cell stage of *A. nanus,* C and P_4 migrate over the D cell, and thus switch places before the onset of gastrulation (Skiba and Schierenberg, 1992).

Skiba and Schierenberg (1992) postulated that the configuration in *A. nanus* is the ancestral state and that the polarity reversal observed in the rhabditids is an evolutionary modification on the '*A. nanus'* like configuration, in order to avoid the compensatory migrations and to speed up the cell cycle periods and thereby the developmental speed. However recently, Lahl et al. (2003) observed reversal of polarity in P_2 and P_3 in several species of the Plectidae, an outgroup of the Rhabditida (Fig. 5.2) (Blaxter et al., 1998; De Ley and Blaxter, 2002). Also

descriptions of the early development of members of other families from clade I, II and III suggest the presence of a small germline precursor at the ventral side that contacts the intestinal precursor (Malakhov, 1994) (Fig. 5.2). Based on the observations in the Plectidae and the other families one can conclude that this reversal of polarity in the germline, and the maintained contact between the germline and the endodermal line found in *C. elegans, P. marina* and *Rhabditophanes* sp. is the ancestral state in the phylum Nematoda. Consequently, the partial reversal of cleavage polarity in the germline found within clade V (*R. dolichura, R. belari*), and clade IV (*H. gingivalis*) and the lack of reversal of polarity in *A. nanus* are derived states and must have evolved independently from the ancestral state in the different branches.

Examples outside the nematodes suggest that the contact between the germline and the gut is a common feature throughout the animal kingdom and is likely to be required for normal germline development. In many other species, such as *Drosophila*, *Xenopus* and mice, the primordial germ cells associate with the hindgut during gastrulation and migration of the germ cells to the gonads (Wylie, 1999). In the late embryonic development of *C. elegans*, the two primordial germline cells project lobes into two intestinal cells, possible for nourishment of the germ cells (Sulston et al., 1983).

One would expect that a derived character has a more efficient design in order to withstand natural selection. In the case of *H. gingivalis* and *A. nanus*, this is not clear. Why would the polarity of the germline be changed, when this change is neutralised by compensatory migrations? A possible explanation would be that the transient contact between EMS and C in *A. nanus* (Fig. 5.1N) is necessary for a communication signal between EMS and C. Wiegner and Schierenberg (1998, 1999) found clues for possible communication between EMS and C in the *A. nanus* embryo. They performed ablation experiments on the early blastomeres of the *A. nanus* embryo, which revealed that these early blastomeres have regulative capacity: eliminated cells can be replaced by its posterior neighbour. So, the somatic blastomeres are able to perform the fate of different blastomeres. This is in strong contrast with *C. elegans,* where the loss of early blastomeres is not compensated by others (Sulston et al., 1983). Wiegner and Schierenberg (1999) presented a model where the fate of each multipotent blastomere is restricted by

specific reciprocal inhibitory cell-cell interactions. In the early 3-cell stage, both AB and EMS are able to perform an AB (1° fate) and EMS (2° fate) like fate (Fig. 5.1 M). AB probably produces an inhibitory signal to EMS that restricts the 1° fate to the AB cell. In the 4-cell stage, AB and C both have the potential to perform the EMS fate (Fig. 5.1N). EMS probably produces an inhibitory signal that inhibits the EMS fate in AB and C thereby restricting the EMS fate to EMS alone. These signals can be transduced through surface bound molecules or through cytoplasmic bridges. Thus, a cell-cell contact between EMS and C is needed to inhibit the EMS fate in C. The reversal of the polarity of P_2 in the *A. nanus* embryo in comparison with the ancestral situation was probably a necessary step in order to establish contact between EMS and C. But why also the polarity of the division of P_3 into D and P_4 is also reversed is not clear from the model of Wiegner and Schierenberg (1999). Perhaps the mechanism to reverse the polarity of P_2 and P_3 is lost completely and the polarity of the germline is determined by a single par-like mechanism. Alternatively, other transient contacts may be needed for possible other inductions that have not been uncovered yet.

Here I propose that the reversed polarities found in *H. gingivalis*, *R. dolichura, R. belari* and *A. nanus* are correlated with the slower development of these species. Firstly, there is an inversed correlation between the division rate of the somatic cells and the division rate of the germline. In slowly developing species the germline divides faster relative to the somatic line (Table 3.1; for a more detailed discussion see 5.1.2). More specifically, in slowly developing species, the germline divides faster than the endodermal line. Secondly, this relative faster division rate of the germline relative to the endodermal line is correlated with variations in the reversal of polarity. In *H. gingivalis*, *R. dolichura, R. belari* and *A. nanus,* P_2 divides before EMS and there is also a variation on reversal of polarity in the germline (Table 5.1; Fig. 5.1 I, N). While in *C. elegans, P. marina, R. terricola* and *Rhabditophanes* sp. P_2 divides after or during the division of EMS and there is complete reversal of polarity resulting in the strict coupling of the endodermal line and the germline (Table 5.1; Fig. 5.1 D,E). In *C. elegans,* the regulation of the divisions of the endodermal line and germline is strictly coupled through the *mes-1* mechanism (Fig. 5.1 A-D). Possibly, in *H. gingivalis*, *R. dolichura, R. belari* and *A. nanus*, where P_2 divides before EMS, this strict

coupling mechanism between endodermal and the germline is relieved, which allowed variations in the reversal of polarity in the germline. This uncoupling between endodermal line and the germline is probably necessary to allow a faster division rate in the germline relative to the somatic line.

The variations in reversal of polarity are found in the different clades (only reversal of P_2 in *R. belari* and *R. dolichura* (Rhabditidae), only reversal of P_3 in *H. gingivalis* (Panagrolaimidae) and reversal of P_2 and P_3 in *A. nanus* (Cephalobidae)) probably reflect the parallel evolution of this trait in these different clades from the ancestral configuration with complete reversal of polarity, which is found in the taxa outside clade IV and V. In all three clades there are fast developing and slowly developing species.

This hypothesis has far reaching implications. If one assumes that the ancestor of clade IV and V has a relatively fast early development with reversal of polarity and that in the different clades there are evolutionary lines towards slower development, one must conclude that the regulative fate specification mechanism found in *A. nanus* has evolved secondarily as a derived character in this clade.

5.1.2. Division sequence

In the three species studied here, the differences in division sequence of the founder cells are mainly caused by the differential division rate of the germline relative to the somatic cells (Table 3.1). Skiba and Schierenberg (1992) observed similar variations in division sequence of the germline in several *Rhabditis* species, *Panagrolaimus* sp. and *A. nanus.* They observed a correlation between the division rate of the germline and the speed of development. In slowly developing species, the germline divides faster relative to the somatic cells. As a result, the primordial germline precursor P_4 arises in an earlier cell stage. They postulated that in slower developing species, P_4 arises earlier in development in order to preserve the germline quality (Skiba and Schierenberg, 1992). In the species studied here, this correlation between speed of development and division rate of the germline is not so clear. Firstly, although the early development of *Rhabditophanes* is little slower than *C. elegans* (1.2 times), P_4 arises earlier in *Rhabditophanes* sp. than in *C. elegans* (in 15-cell stage and in 24-cell) (Table 3.1).

However, this large difference can be explained by only a small temporal shift in the division of P_3, from before 8AB in *Rhabditophanes* sp. to during 8AB in *C. elegans*. Secondly, the early development of *P. marina* is two times slower than in *Rhabditophanes* sp. Still in both species, the germline precursor P_4 arises at the 15-cell stage (Table 3.1). Maybe there are other yet undiscovered factors that influence the division rate of the germline.

5.1.3. Developmental tempo

The three species studied here do not only differ in total developmental speed but also in the pattern of cell-cycle length (Fig. 3.6). Laugsch and Schierenberg, (2004) proposed a model that explains the variations in cell-cycle lengths with the differential availability of maternal vs. zygotic material necessary for cell division. They observed four patterns (Fig. 5.3). Firstly, in *C. elegans*, cell cycle length increases slowly, which correlates with a slowly diminishing amount of maternal supply. Secondly, in contrast with *C. elegans*, in *A. nanus*, which has very little maternal supply, the slow cell cycles speed up during the course of development, indicating an increasing amount of zygotic transcripts. In between, in species like *R. belari*, the cell cycle length first slows down and then accelerates again, indicating a balance between depleting maternal resources and increasing zygotic transcripts (Laugsch and Schierenberg, 2004). An extreme case is found in *R. dolichura*, where there is a cleavage pause during development, indicating a possible time gap between the depleting maternal supply and the start of zygotic transcription (Laugsch and Schierenberg, 2004).

In *H. gingivalis*, the cell cycle length of AB slows down, following the model of *C. elegans*, although these cycles are twice as slow in *C. elegans*. In *Rhabditophanes* sp. and *P. marina*, the cell cycle length of AB shows a small peak in, after which the development shortly speeds up again. These species follow the pattern of *R. belari*. The moment of this peak possibly indicates a bending point between depleting maternal supplies and increasing zygotic transcription (Fig. 5.4). In *Rhabditophanes* sp. this peak occurs one cell cycle later than in *P. marina*, *R. belari* and *R. dolichura* (8AB in comparison with 4AB), indicating that there is possibly a larger contribution of maternal supply in *Rhabditophanes* sp. The contribution of the maternal supply to the early development can be determined by

blocking the embryonic transcription with α-amanitin. Then the early embryo solely depends on the maternal supply for its development. In *C. elegans* with strong maternal control, the embryo is able to develop until the 100-cell stage without embryonic transcription (Edgar et al., 1994), while in *A. nanus* the embryonic development stops in the 5–cell stage, indicating only a minor contribution of maternal products to the early cleavages (Wiegner and Schierenberg, 1998).

After the 16AB stage, the cell cycles of the AB generations in *P. marina* and *Rhabditophanes* sp. slow down again. It is not known whether this is a general trend, since no cell cycle data later than 16AB is available for the other species studied in Laugsch and Schierenberg (2004).

5.1.4. Bilateral symmetry.

In the species studied here, the founder cells establish bilateral symmetry in their first (MS, C, D) or second division (AB, E) generating bilateral symmetric cell lineages (Fig. 3.6). However this symmetry is not always obvious after the first division, since the founder cells divide with a main anterior-posterior polarity. For example, in *P. marina, Rhabditophanes* sp. and *C. elegans,* MSa and its descendants lie more anteriorly than their bilateral equivalents of MSp. Also C divides primarily in anterior posterior direction; the symmetry between Ca and Cp is established later in development by cell migrations. Lahl et al. (2003) called this phenomenon weak bilateral symmetry, in contrast with the strong bilateral symmetry found in the embryonic development of Plectidae, where all the founder cells have strict left-right symmetry from their first or second division. The species studied here have an intermediate form of bilateral symmetry. MS divides more anterior posterior as in *C. elegans*, while C divides strictly in left-right direction, as in the Plectidae. Lahl et al. (2003) mentioned *A. nanus* as an intermediate form with a strict symmetry between the two daughters of C. Also from the descriptions of the early development from species in the basal clades I and II (Malakhov, 1994),

The weak bilateral symmetry and compensatory cell migrations in *C. elegans* is in contrast with its fast development. One would expect the opposite, that in *C.*

elegans divisions are optimised to put the cells at the place where they are needed to minimize migrations (Houthoofd et al., 2003).

5.1.5. Gastrulation.

Start of gastrulation is delayed in *H. gingivalis* and *Rhabditophanes* sp. to the end of 2E stage and 4E stage. This is in contrast with *P. marina* and *C. elegans*, where the two E daughters first migrate inwards to final position and then divide into four cells. Also in the embryonic development of the animal parasitic nematode *Rhabdias bufonis*, the two E cells divide before the onset of gastrulation (Spieler and Schierenberg, 1995). A molecular phylogenetic analysis places *R. bufonis* as sister taxon of the clade of *Rhabditophanes* sp. and *Strongyloides* (Dorris et al., 2002). The family of *H. gingivalis,* Panagrolaimidae, is grouped together with the (*R. bufonis, Rhabditophanes* sp.*, Strongyloides*) clade and is called the IVa clade (next to the IVb clade which consists of the Cephalobidae and Tylenchidae). Based on the limited data of these three species, the delayed gastrulation seems to be a synapomorphy for clade IVa. Within these species there is a difference in order of cells that undergo gastrulation: in *Rhabditophanes* sp. Ep cells gastrulate before Ea, in *H. gingivalis* first Ea, then Ep gastrulation. In *Plectus* species, the E gastrulates as a single cell and divides afterwards (Lahl et al., 2003). Also in *Plectus* the division of E is delayed until after the division of 8AB, as in *H. gingivalis*. The timing of gastrulation and the number of intestinal precursors that gastrulate vary widely between different species. As a consequence, the cellular environment of the gastrulating intestinal precursors in those species is different. However, that does not seem to have many implications on the gastrulation process. In *C. elegans*, gastrulation is independent from outer signals of the surrounding cells MS or P_4. Solely apical constriction of E cells that pulls MS and P_4 together. The loss of contact between P_4 and E-cells in *H. gingivalis* has no effect on gastrulation, assuming a similar gastrulation mechanism in *H. gingivalis*.

5.2. THE LINEAGE ORIGIN OF THE ORGANS VARIES FROM HIGHLY CONSERVED TO HIGHLY VARIABLE

The embryonic development of *P. marina, Rhabditophanes* sp. and *H. gingivalis* is as invariant as in *C. elegans*. Comparison of the tissue configuration of these species with this in *C. elegans* shows a variable degree of conservation. The intestine, the primordial gonad and the body muscles are highly conserved in the four species, while the pharynx, the epidermis and the nervous system have a more variable configuration.

5.2.1. Intestine

Two intestinal patterns have been found in the species studied here. The intestinal development in *P. marina* and *Rhabditophanes* sp. follow a similar a route as in the *C. elegans* embryo. While *H. gingivalis* has a different intestinal pattern, in which 18 intestinal cells are formed and two precursors undergo cell death. However the result in these two patterns is the same: a bilateral symmetric embryonic intestine consisting of 9 subsequent pairs of cells. How do these two intestinal patterns relate to each other, which one is the ancestral one and which one the derived one and a possible evolutionary explication for the observed differences is discussed in detailed here.

5.2.1.1. Position of the mesoderm precursors may constrain the establishment of bilateral symmetry in the P. redivivus *pattern*

According to the phylogenetic analysis, a new variant of gut formation evolved within the family of the Panagrolaimidae. However, these two distinct patterns lead to the same result: an embryonic bilateral symmetric intestine, built up by nine consecutive rings.

Here I propose a hypothesis based on the observations made in this study that can explain the difference between the two intestinal patterns. The establishment of bilateral symmetry in the Ea lineage in the *P. redivivus* pattern is possibly constrained by the position of the MSap and MSpp descendants during their concerted gastrulation. Since Ea is positioned in between MSap and MSpp during

its gastrulation (Fig. 4.6A, Fig. 4.7A), no left-right division of Ea would be possible. This is in contrast with its posterior daughter Ep, which has no extra neighbors, but the lateral AB cells, during its gastrulation. For the same reason could the bilateral symmetry be established secondarily by migration of the daughters to the left and right position or by a subsequent left-right division of the daughters. Instead Eaa and Eap divide once more asymmetrically in anterior-posterior direction (Fig. 4.6C, Fig. 4.7C). The bilateral symmetry is established by migration of the anterior daughter of Eaa, Eaaa, to the left side of the embryo, when the E descendants have reached a more dorsal position than the MS descendants (Fig. 4.6D, Fig. 4.7D). In the *C. elegans* pattern, Ea migrates inwards before MSap and MSpp, so there would be no such constraint of MSap and MSpp on the division of Ea. Ea is positioned dorsally of the MS descendants and therefore can divide left-right to establish bilateral symmetry in the four cell intestinal primordium (Fig. 4.7H).

However, one cannot exclude another possibility, that an intrinsic change in the lineage and division axes of the intestinal precursors is the cause of the change in spatial configuration of the embryonic intestine. A more experimental set up could elucidate this problem. For example, one can test the possible spatial constraint of the MSap, and MSpp and their descendants on the development of the intestine, by killing the precursor cells MSa and MSp in a species with the *P. redivivus* pattern with laserablation. If after ablation of MSa and MSp, the lineage of E transforms again to the *C. elegans* pattern, then MSap and MSpp are the cause of the transformation in the intestinal pattern. If no change in the intestinal pattern is observed, then there is no spatial constraint of MSap and MSpp must be an intrinsic cue in the E-lineage.

5.2.1.2. Programmed cell deaths are indications of a reprogramming of the intestinal cell lineage

Also the occurrence of the cell deaths in the *P. redivivus* pattern can be explained by the proposed hypothesis. The two subsequent antero-posterior division in the Ea lineage result in a row of 4 antero-posteriorly orientated cells. The asymmetrical division and subsequent cell deaths of the posterior daughters of Eaa and Eap probably prevent the primordial intestine to become too long and may facilitate the secondary establishment of symmetry. The loss of Ea(a/p)p,

which produce four cells or two rings in the *C. elegans* pattern, are compensated by additional cell divisions of Ep(l/r)pa, and the antero-posterior orientation of the division of Ea(a/p)aa.

The use of programmed cell deaths in reprogramming cell lineages of nematodes is widespread. For example, cell deaths are used to refine stereotypical sublineages that are used repeatedly, for example in the ventral nerve cord of *C. elegans* (Sulston and Horvitz, 1977; White et al., 1986) and vulval cell lineages in *Pristionchus pacificus* (Sommer and Sternberg, 1996). In nematodes with a monodelphic female gonad (only one anterior arm) the monodelphy results from the programmed cell death of one cell, the posterior distal tip cell, that induces the growth of the posterior gonadal arm in didelphic species (Sternberg and Horvitz, 1981; Felix and Sternberg, 1996).

5.2.1.3. Evolutionary modification that leads to the same result

Gould (2002) mentioned that oddities or imperfections, making no sense as an optimal design in a current context, could be explained as holdovers from the past state. He makes the analogy with letters in a word that are still retained in the spelling, but become useless in the pronunciation. These can serve as a clue in seeking for its derivation. Here, the cell deaths in the intestinal cell lineages of *P. redivivus* and *H. gingivalis* can be considered as developmental anomalies and betray evolutionary signs from the past, more precisely the modification of the establishment of the bilateral symmetry in the intestine. Why are these cells generated and discarded immediately after their birth? Another solution would be discarding the preceding cell division. However, these cell divisions and generation of these cells are retained in evolution, but becomes useless in the development of the intestine.

The evolutionary modification of the intestinal lineage however has no effect on the ultimate design of the embryonic intestine, a bilateral symmetric intestine of nine rings. Other examples of how developmental mechanisms can evolve without affecting other aspects of development have already been described in nematodes and other animals. Goldstein et al. (1998) showed that a new axis specification mechanism has evolved in the ancestors of relatives of *C. elegans* without affecting further development. Comparative analysis showed that the fate

specification of early blastomeres in different nematodes varies considerably without influencing the resultant structure of the nematode (Schierenberg, 2001). Another class of examples is the modification of larval growth in closely related species of sea urchins without affecting the adult morphology (Wray, 1994). This can be found throughout the animal kingdom, in sea urchins, molluscs, ascidia and vertebrates (reviewed in Raff, 1996).

This study has focused on the intestinal development of species from clade IV and V according to the molecular phylogeny of Blaxter et al. (1998). Observations of the early development of species from clade I and II also indicate the presence of a single intestinal precursor cell (Malakhov, 1994; Voronov and Panchin, 1998). Despite this generality in nematodes, important differences indicate different specification mechanisms of this single precursor. In species of clade I this precursor cell arises from the anterior blastomere in the 2-cell stage, instead of the posterior cell in clade IV and V (Malakhov, 1994). In *Enoplus brevis* (Enoplidae, clade II) a single intestinal precursor arises in the 8-cell stage in an otherwise indeterminate cleavage pattern where the other cells remain undetermined (Voronov and Panchin, 1998). And Schierenberg (2005) observed a different mode of gastrulation in *Tobrilus diversipapillatus* (Tobrilidae, clade II), where a large blastocoel is formed and cells migrate into this cavity after the 64-cell stage. Further investigations on developmental mechanisms of species in these clades would help us fully understand the diversity of developmental mechanisms of the nematode intestine and more experimental studies, like cell ablation experiments, would teach us more how these mechanisms work.

5.2.2. Gonad

Also very conserved is the behaviour of the primordial germ cells (PGC). In the three species the germ line cells are closely associated with the cells of the fifth intestinal ring. Electron micrographs of a *C. elegans* embryo in the second half of the embryogenesis show that the germ cells protrude large lobes into the two intestinal cells (Sulston et al., 1983). Probably the germ cells are also nursed by the intestine until their attachment to the somatic gonadal cells, since the latter are essential for the survival and the further development of the gonad (Kimble and White, 1981). Also in the enoplid *Enopus demani* two large primordial germ cells

are found closely associated with the intestine, but it is not clear from which blastomere they come (Malakhov, 1994). Also in many other phyla, the PGC's are closely associated with the early endoderm at the vegetal pole of the egg (e.g. in Amphibia) and the endoderm is necessary for the correct migration of the PGC's to the somatic gonads (*Drosophila* (Warrior, 1994; Jaglarz and Howard, 1995), Amphibia (Bounoure, 1934; Ressom and Dixon, 1988; Kloc et al., 1993), Mammalia (Heath, 1978)). The fact that these two tissues perform the two most primitive Metazoan functions – feeding and reproduction – could explain the strong conservation and association of them in *P. marina*, *Rhabditophanes* sp., *H. gingivalis* and *C. elegans* and throughout the animal kingdom.

The precise number of PGC's seems not to be constrained during the course of the evolution of embryonic development of nematodes. The *P. marina* (clade V) and *H. gingivalis* (clade V) embryo both have two PGC's, just like *C. elegans* (clade V) (Sulston et al., 1983) and *R. terricola* (Laugsch and Schierenberg, 2004). Also in descriptions of the embryonic development of *Enoplus demani* (Enoplida, clade I) and *Eustrongylides excisus* (Dioctophymida, clade II), two PGC's have been observed in their embryos (Malakhov, 1994). On the other hand, in *Rhabditophanes* sp. (clade IV) the two P_4 cells have an extra division to form four PGC's. And finally, Laugsch and Schierenberg (2004) showed that *Rhabditis belari* (clade V) and *A. nanus* (clade IV) have only one PGC. From this limited amount of data, one can conclude that two PGCs is probably the ancestral state, since in all clades nematodes are found with two embryonic PGCs. As a consequence, the one PGC state must have evolved twice in clade IV (in *A. nanus*) and V (in *R. belari*) and a duplication of PGC's must have occurred with clade IV (in *Rhabditophanes* sp.). However, more developmental data from other species in the different groups should be collected to confirm this.

It is not clear if the presence of four PGC's has any implication on the further post-embryonic development of the gonad in *Rhabditophanes* sp., since this species has a didelphic gonad with two gonadal arms, and a similar number of cells as in *C. elegans*. In *C. elegans* the two PGCs divide continuously in the different juvenile stages (Kimble and Hirsch, 1979). The extra division of the two P4 cells can be seen as a heterochrony from J1 to the embryonic phase, with possibly no effect of the further development of the gonad. However the post-

embryonic cell lineage of the gonadal cells of *Rhabditophanes* sp. should elucidate this issue.

5.2.3. Pharynx

In *P. marina* and *Rhabditophanes* sp., ABp contributes pharyngeal cells (Fig. 4.12). In *C. elegans*, an interaction from P_2 to ABp prevents the ABp lineage from responding to an MS-derived pharynx-inducing signal to produce pharyngeal cells (Hutter and Schnabel, 1994; Mango et al., 1994; Mello et al., 1994; Moskowitz et al., 1994). This could mean two things. First the P_2 signal is absent in *P. marina* and *Rhabditophanes* sp. or second, later signals induce secondary production of pharyngeal cells. If this signal in *C. elegans* is absent, then ABp adopts an ABa-like fate (Mello et al., 1994). However, in *P. marina* and *Rhabditophanes* sp. only a few ABp cells adopt a pharyngeal fate in an AB lineage that very much resembles the ABp lineage of *C. elegans*. So, the pharyngeal cells in ABp are most likely induced secondarily, later in embryonic development.

The similar number of pharynx cells between the four species does not reflect the differences in the adult morphology of the pharynx in the four species. *H. gingivalis* has only one bulbus in the pharynx, while the other species have two bulbi. The definitive shape of the pharynx is formed during the second part of embryonic development after most of the cell divisions and after body muscle contractions.

5.2.4. Body muscle

Both, *P. marina* and *C. elegans* have the same 81 body muscle cells in a similar configuration. Also in the *Rhabditophanes* sp. embryo 82 body muscle cells are present in a similar configuration. Apparently the program for muscle formation is strongly conserved. Probably not much variation in the course of evolution was possible for the proper functioning of the muscles. There is no obvious explanation for this conservative pattern. Nevertheless *mes-1* mutants of *C. elegans* in which P_4 forms extra body muscles cells seem to function well (Strome et al., 1995). Also *C. elegans* embryos, lacking 20 of the 81 embryonic body wall muscle cells by

ablation of the D blastomere, become viable adults, indistinguishable from wild type animals (Moerman et al., 1996).

5.2.5. Epidermis

The epidermis is a more variable tissue although some basic patterning in the four species compared here is similar. More variation between the species is found in the epidermis cells that are specified later in the last division rounds. It seems that there is a conserved pattern of epidermis formation that can be adapted to a specific epidermis tissue by adding or deleting epidermis cells in the last division rounds of the cell lineage.

5.2.6. Nervous system

The nervous system is the most variable tissue between the four species. A possible explanation would be that the configuration of the nervous system is adjusted to specific functional needs, by transforming cells to neurons that originate close to their final position, rather than by moving the existing neurons to a new position. One could expect that cell deaths exhibit the same plasticity. The high degree of homology of the cell deaths between *P. marina* and *C. elegans* contrasts somewhat with the lower degree of similarity in the nervous system. Although rewiring of the pharynx could be a possibility, there is a high morphological similarity between the pharynxes of both species, making this rather unlikely.

5.3. MONOCLONAL VS. POLYCLONAL FATE SPECIFICATION AND POSSIBLE INFLUENCE ON SPEED OF DEVELOPMENT

5.3.1. Variations in fate distribution within a conserved pattern

Based on this comparison of four nearly complete embryonic cell lineages, we conclude that there is a conservation of a '*C. elegans*' like polyclonal cell lineage with strong left-right asymmetry (Fig. 3.12) in clade IV and V of the phylum Nematoda. This developmental strategy is very robust, despite the large evolutionary distance and the great genomic diversity in these clades (Parkinson et al., 2004; Makedonka et al., 2005). We only observed variations in the fate distribution of cells at the end of the cell lineage between individual species. The four species have high lineage similarities (93%-95%), but lower fate similarities (76%-85%). This means that cells are formed by the same division pattern, but get a different fate. This fate similarity is also unequally distributed over the different founder cells. The potential of the founder cells originating from the posterior blastomere in the 2-cell stage (MS, E, C, D and P_4) have a high similarity of 90% and more. Most of the variation in fate distribution was found in the anterior blastomere, the AB founder cell. Still, the variations in fate between equivalent cells of the AB lineage between *P. marina, C. elegans, Rhabditophanes* sp. and *H. gingivalis* are found within a conserved pattern.

The cells in AB are polyclonally specified; cells from one tissue are formed by lineally unrelated cells. For example, neurons are formed throughout the cell lineage in all AB great-granddaughters. But in all four species studied here, this polyclonal fate distribution has a similar distribution over the 8AB great-granddaughters (Fig. 4.12). This asymmetric, polyclonal fate distribution of AB in *C. elegans* is regulated by a series of four Notch inductions, which results in a specific fate distribution for each of the 8 AB great-granddaughters (Gendreau et al., 1994; Moskowitz et al., 1994; Hutter and Schnabel, 1994, 1995a,b; Moskowitz and Rothman, 1996). First, in the 4-cell stage of the *C. elegans* embryo, the potential between the two daughters of AB, ABa and ABp is differentiated by an inductive signal of germline precursor P_2 to ABp. As a result the distribution of the epidermal precursors arising from ABp is reversed along the AP axis relative to

those arising from ABa. In ABa neuronal cells are formed in the anterior granddaughters ABala and ABara, while epidermal cells are formed in the posterior granddaughters ABalp and ABarp (Figure 3). In ABp however, neuronal cells are formed in posterior granddaughters ABplp and ABprp and epidermal cells in the anterior granddaughters ABpla and ABpra (Fig. 4.12). This pattern is adapted by a second induction in the 12-cell stage, where a signal from MS to ABalp and ABara induces pharyngeal potential in those cells. We observed a similar fate pattern in the 8AB descendants of *Rhabditophanes* sp., *H. gingivalis* and *P. marina* although in varying percentages (Fig. 4.12, Table 4.4). The pattern is illustrated here with the *Rhabditophanes* results but is similar for the other species. Epiderm cells are mainly formed in the posterior daughters of the ABa lineage by ABalp and ABarp (30/32) and in the anterior daughters of the ABp lineage by ABpla and ABpra (37/40). Pharyngeal cells are formed mainly by ABalp and ABara (62/63).

A third and fourth Notch interaction influences subsets of those 8AB precursors of the *C. elegans* embryo. At the 24-cell stage, an interaction occurs between ABalap and ABplaa (Hutter and Schnabel, 1994, 1995a,b; Moskowitz and Rothman, 1996). As a consequence, the anterior daughter ABplaaa develops differently from their bilateral homologue ABpraaa and forms left head precursors that are bilateral symmetric with the ABarpap descendants that form right head precursors. The same symmetry breakage between the equivalent cells ABplaaa and ABpraaa has been observed in *Rhabditophanes* sp. and *H. gingivalis*, whereby ABalap contacts ABplaaa but not ABpraaa (Fig. 5.5A). Moreover, the ABplaaa descendants lie bilateral symmetric with the ABarpap descendants to form left and right head precursors (Fig. 5.5B). And last, a fourth asymmetric interaction occurs between MSap and ABplpa (Hutter and Schnabel, 1995a; Moskowitz and Rothman, 1996). As a result, one of the ABplpapp descendants, ABplpappaap, produces the excretory cell. Similarly, in *Rhabditophanes* sp., there is contact between MSap and ABplpa (Fig. 5.5C) and one large excretory cell is formed by the same cell as in *C. elegans* (Fig. 5.5D).

Although the underlying cell specification mechanisms have not been uncovered in *P. marina, Rhabditophanes* sp. and *H. gingivalis*, the result is the same: an asymmetric cell lineage with polyclonal fate distribution. Sulston et al.

(1983) concluded that the most striking finding about the embryonic cell lineage of the *C. elegans* was its complexity. Also Schnabel et al. (1997) acknowledged that there seems to be no underlying rules to specify tissues. Then what is the evolutionary advantage of such a seemingly complex polyclonal cell specification? Therefore I will compare this type of cell specification with its opposite mechanism: the monoclonal cell lineage. In monoclonal cell lineages, cells from one tissue are formed by lineally related cells.

5.3.2. Polyclonal vs. monoclonal cell lineage

In polyclonal lineages, the fate of the terminal cells is correlated with its position in the embryo instead of its position in the lineage. Lineally related cells have a similar position instead of a similar cell fate. So, cells are made close to the place where they are needed, thereby minimizing the need for migrations. In monoclonal cell lineages, the fate of the cells is correlated with its position in the cell lineage instead of its position in the embryo. Lineally related cells have the same fate instead of position. After their specification, these cells migrate to their final position. A clear example that illustrates the difference between the monoclonal and polyclonal specification can be found in the Caa-lineage (fig. 5.6A-C). In *H. gingivalis* the Caa-lineage forms only epidermal cells (fig. 5.6A). Those cells migrate to their final position. In the Caa-lineage of *P. marina, Rhabditophanes* sp. and *C. elegans,* two cells are specified into tail neurons (fig. 5.6B,C). In *H. gingivalis* those neurons are formed in the AB-lineage in the anterior part of the body and later migrate to the tail region (fig. 5.6D). This migration is not necessary in *P. marina, Rhabditophanes* sp. and *C. elegans* by forming those two nerve cells in the Caa-lineage in the posterior part of the embryo close to their final position in the embryo (fig. 5.6E,F). In *H. gingivalis*, cells are specified in clusters of monoclones, which arrive in different places of the embryo and as such have to migrate to their final positions. Thus in *H. gingivalis*, lineally related cells have the same fate, but arrive at a different position. While in the polyclonal pattern of *P. marina, Rhabditophanes* sp. and *C. elegans*, the cell lineage is arranged so that cells originate close to their final positions. Here, lineally related cells have a similar position but a different cell fate.

Stent (1985) already recognized the monoclonal and polyclonal patterns and called these respectively typologically and topographically hierarchic schemes. In invertebrate species as nematodes and leeches, where the embryos have a limited number of cells and cell migration plays a minor role, two lineally related cells have a similar position rather than a similar cell fate (Weisblat et al., 1984; Shankland and Weisblat, 1984*)*. In vertebrates however, where cell numbers range in the millions, cell migration plays a prominent role. For example, in vertebrates, neuron precursor cells are formed in the neural crest and later migrate to their ultimate position (LeDouarin, 1980).

A cell lineage with monoclonal cell fate distribution suggests a simple specification mechanism. Possibly, less specification events are needed, no matter which mechanism is used (distribution of determinants, differential genetic expression or induction signals between cells). As such, the decision can occur once at the precursor cell of the clone. A disadvantage of this strategy is that those cells have to migrate to their final position. For example the tail neurons in *H. gingivalis* originate anteriorly in the AB cell, from which all neurons originate, and migrate posteriorly to arrive in the tail (Fig. 5.6D). This would suggest a cross-traffic of terminal cells and an accompanying complex signalling mechanism to get these cells to the correct position on time and thereby slows down the embryonic development. Furthermore, this poses a risk in an embryo with a limited number of cells. There is no flexibility, for example if the leading epidermal cells do not arrive at their correct position on time, the ventral enclosure of the epidermis could fail which causes a preliminary arrest of the embryonic development (Williams-Masson et al., 1997). On the contrary, in vertebrates where clones of millions of cells are made, loss of one or more cells could easily be compensated for by others (LeDouarin, 1980; LaBonne and Bronner-Fraser, 1999).

A cell lineage with polyclonal cell fate distribution could allow a faster embryonic development since cells are born close to the place where they are needed and thereby reducing the need for cell migrations. It is unlikely that this strategy alone would suffice to explain the fast embryonic development in *Rhabditophanes* sp. and *C. elegans*. This strategy is only one of the many possible strategies for fast embryonic development. Supply of maternal gene products enhances the speed of the early development (Skiba and Schierenberg, 1992; Wiegner and Schierenberg,

1998) and the *clk-1* gene in *C. elegans* regulates physiological growth rate in embryos (Wong et al., 1995). The strategy of polyclonal specification pattern only influences the length of the period up to organogenesis, in the period where cells are formed, specified and migrate to their final position. The strategy is correlated only with this period and not with the whole generation time. It is possible that there exists a nematode with a slower first part of embryogenesis but with a faster overall development. There are other genetic mechanisms that regulate the length of post-embryonic development. For example, in *C. elegans*, heterochronic *lin* genes regulate the division timing of certain blastomeres (Slack and Ruvkun, 1997).

Disadvantage of the polyclonal specification strategy is the likely complex specification mechanism, where almost every cell has to be specified individually. In an embryo with limited number of cells, if a cell is wrongly specified, a wrong cell arrives at the wrong place, without the possibility for correction.

5.3.3. Complexity of polyclonal cell lineages

So, two factors play a possible role in the difference between monoclonal and polyclonal cell lineages. First, there is the complexity of the specification mechanism, which is probably simple in monoclonal cell lineages and complex in polyclonal cell lineages. A second factor is the need for cell migrations to put cells at their terminal positions, which is high in monoclonal cell lineages but low in polyclonal cell lineages. These two factors have been tested by evolutionary modelling explained below.

First we need to define and quantify the complexity of a cell lineage. Based on the cell lineages of *P. marina, H. gingivalis* and *C. elegans,* Azevedo et al. (2005) designed a measure of lineage complexity defined as the length of its shortest algorithmic description. In a first step, the cell lineages are coded as a list of rules each corresponding to a cell division: X→(Y,Z) (cell X divides into cells Y and Z). Equivalent rules, which form the same set of cells, are eliminated until we get a list of reduced rules encoding a complete non-redundant description of the lineage. The lineage complexity C is then defined as the number of reduced rules expressed as a proportion of the total number of cell divisions (maximum possible

number of rules for a lineage of the same size). The number of reduced rules estimate the minimum number of intermediate states required to generate a given distribution of terminal cell fates. The more reduced rules are needed to describe a cell lineage, the more complex this cell lineage is. These reduced rules can be seen as developmental modules, programmed to specify a certain differential fate distribution in each division. In a next step we measured the complexity of the complete embryonic cell lineages of *C. elegans*, *P. marina* and the partial cell lineage of P_1 of *H. gingivalis*. These lineages show complexities of 35%, 38 and 33% respectively. This means that only 33%-38% of the cell divisions give a unique fate distribution in these cell lineages. Although these polyclonal cell lineages look complex and chaotic, there is order in it, in a way that reduced rules or developmental modules are reused. So nematode cell lineages are not that complex as they look like in terms of specification mechanisms as Sulston et al. (1983) and Schnabel et al. (1997) implied. Moreover, to test if nematode cell lineages were simpler than could be expected, we compared the complexities of the cell lineages with the complexities of random generated cell lineages with the same cell number and fate composition. We found that the real cell lineages are 26-45% simpler than could be expected by change.

Nematode cell lineages might have evolved towards simpler lineages with less reduced rules, but are they as simple as they might be? Therefore, we performed evolutionary simulations to search for simpler lineages with the same composition. At each generation, a population of 100 variant cell lineages was produced and the simplest daughter was allowed to found a new generation of cell lineages. These evolutionary simulations generated cell lineages that were 10-18% simpler than the real cell lineage after 20,000-50,000 generations. Thus although nematode cell lineages are simple, they are not a simple as they might be.

This is because reduced complexity is not the only factor that shapes cell lineages. Monoclonal cell lineages may be even simpler with a minimum of reduced rules. But in a monoclonal cell lineage, cells are specified according to their lineage position. So, after their birth they have to migrate to their final position. This can be a major developmental constraint in an embryo with few cells and it slows down development. To test the effect of this second factor in the evolution of cell lineages, we repeated our evolutionary simulations but with an

extra spatial constraint factor on the position of the cells in the embryo. We found that dependent on the severity of the constraining factor, the response in the simulations decreased by 1.9-5.7%. Here we show that the nematode cell lineages are almost as simple as the simplest evolvable under strong constraints on the spatial positions of cells.

These strategies of monoclonal vs. polyclonal cell lineages are but extremes. The embryonic cell lineages of the three species studied here probably represent an evolutionary compromise between minimizing the number of specification events and minimizing the need for migration. Each organism uses a combination of the two strategies.

5.3.4. Polyclonal cell lineage correlated with speed of development?

In the different branches of clade IV and V, there are both slow developing and fast developing species. In the Rhabditidae (clade V), *C. elegans* has a fast development (embryogenesis in 12h at 20°C), while *P. marina* and other *Rhabditis* species have a slower development. In clade IV, *Rhabditophanes* sp. has a fast development in comparison with *H. gingivalis*. Based on the cell lineage of *P. marina* and the partial cell lineage of *H. gingivalis,* we proposed that the polyclonal cell lineages found in *C. elegans* and *P. marina* are linked to a fast mode of development (Houthoofd et al., 2003). In this study, I compared nearly complete embryonic cell lineages of both fast and slowly developing species in these two clades. So, I can make a more clear statement over how this polyclonal cell lineage evolved and if this strategy is linked to the evolution of speed of development in clade IV and V?

There are two possible scenarios for the evolution of the speed of development in clade IV and V. First, the ancestor of clade IV and V could have had a slow, more regulative development, as can be found in *A. nanus* (Skiba and Schierenberg, 1992; Wiegner and Schierenberg, 1998, 1999). This could be consistent with the slow regulative development of species in the outgroup and basal clades I and II. Then fast developing nematodes species with a strict polyclonal cell lineage evolved from this slow ancestor. But then this fast polyclonal cell lineage should have evolved at least twice in the two clades. This is

unlikely, since the polyclonal cell lineage of *P. marina* and *C. elegans* of clade V and the lineage of *H. gingivalis* and *Rhabditophanes* sp. have strong similarities with a high fate similarity, a similar fate distribution in AB and identical secondary symmetries in AB.

A second scenario would be that the ancestor of clade IV and V was a relatively fast developing species with a strict polyclonal *'C. elegans'*-like cell lineage. Then in both clades, more slowly developing species evolved from this fast ancestor, still retaining this polyclonal embryonic cell lineage. Then, the speed of development could be primarily linked to other factors, such as the amount of maternal control (Laugsch and Schierenberg, 2004). This scenario would imply that the regulative early development in *A. nanus* (Wiegner and Schierenberg, 1998, 1999) has evolved secondarily from a mosaic ancestor. Support for an ancestor with no regulative capacities, is that species of the family Plectidae, which are an outgroup of clade IV and V (Blaxter et al., 1998), have no regulative capacities in the early development (Lahl et al., 2003). This also implies that this polyclonal cell lineage has evolved early in nematode evolution before the diversification of clade IV and V and was a prerequisite for the fast development of some of the species in these two clades. Fate transformations within these clades as shown between *C. elegans, P. marina, Rhabditophanes* sp. and *H. gingivalis* would have only a marginal contribution to faster development in faster developing species such as *C. elegans* and *Rhabditophanes* sp..

The nearly complete data set of *H. gingivalis* and the one of *Rhabditophanes* sp. now favour for the second scenario, because the polyclonal cell specification is much more widespread in clade IV and V and is not a highly derived trait that is specifically linked to the fast development of *C. elegans* as Bolker (1995) hypothesised.

5.3.5. Asymmetric cell lineage linked with symmetry breakage in 6-cell embryo

A second common feature between the four species studied here is the conserved asymmetry in the AB lineage. The lineally symmetric AB granddaughters ABal, ABar, ABpl and ABpr give rise to non symmetrical cells and

different tissues (eg compare fate distribution between ABala and ABara in Fig. 4.12). In *C. elegans*, this bilateral asymmetric lineage is the result of a signalling pathway to superimpose the bilateral symmetry of the nematode body plan on an asymmetric 6-cell stage embryo, after the skewed left-right division of ABa and ABp (Sulston et al., 1983; Wood, 1991). Also the studied species of clade IV have this single symmetry breakage event in the early embryo. Although the underlying cell specification mechanisms have not been uncovered in other nematodes, the result is the same: an asymmetric cell lineage that compensates for the asymmetry in the early embryo. We hypothesise that this symmetry-breaking event in nematodes of clade IV-V is a major developmental constraint that shapes the subsequent asymmetric cell lineage in those nematodes. The studies on the early development of nematodes from clade I-III indicate that early embryos in these more basal clades are symmetric (Malakhov, 1994; Voronov and Panchin, 1998; Lahl et al., 2003; Schierenberg, 2005). It remains to be seen however if these symmetric embryos give rise to a symmetric cell lineage.

5.3.6. Conclusion

Based on this comparison of four nearly complete embryonic cell lineages, we conclude that there is a conservation of a '*C. elegans*' like polyclonal cell lineage with strong left-right asymmetry in clade IV and V of the phylum Nematoda. The high fate similarity, the conserved fate distribution in AB and the identical secondary symmetries in AB are indications that this lineage has evolved before the diversification in clade IV and V. We propose that a symmetry-breaking event in 6-cell embryo of nematodes belonging to clade IV-V is a major developmental constraint that shapes the subsequent asymmetric cell lineage in those nematodes. This strong developmental constraint in genetically diverse nematodes is in strong contrast with other phyla, like arthropods and chordates, where a more conserved genetic program gives rise to a large variety of developmental patterns, eg. Hox clusters (reviewed in Peel et al., 2005; Pearson et al., 2005).

5.4. EVALUATION OF COMPARATIVE LINEAGING

After performing a thorough comparative study of four nearly complete embryonic cell lineages of species in clade IV and V, it would be interesting to think about the future of comparative lineaging of nematode cell lineages. Would it be worthwhile to expand the study with embryonic cell lineages of more different nematode species? The answer to this depends on which question one wants to solve.

The original question of this project was to find out if the fast and strictly determined cell lineage of *C. elegans* is a highly derived feature, linked to its fast development or if it is a more general mode of nematode development. A second question was how and when this fast polyclonal mode of development evolved from a slower and regulative development found in the basal clades of the nematodes. From this study, one can conclude that this complex polyclonal cell lineage is conserved within clade IV and V. Since the four species compared in this study have the same asymmetric polyclonal cell lineage, it is likely that the ancestor of clade IV and V already had a "*C. elegans*" like polyclonal cell lineage. So, in order to find nematodes with more ancestral form of development, one has to look for nematodes outside these clades. So, more cell lineage data from other families in clade IV and V, not included in this study, could only confirm the extent of conservation of a polyclonal lineage in clade IV and V or elucidate other developmental mechanisms derived from this ancestral polyclonal lineage. Given the great effort needed to establish an embryonic cell lineage with the current technology - it takes on average one year to establish one with 4D microscopy – it is in my opinion not worthwhile to establish more embryonic cell lineages in these clades. One family, the Cephalobidae, deserves a closer look. In this family a species *A. nanus*, was found with a regulative early development, which is probably a derived feature (see 6.1.1). It would be interesting if this early development has any impacts on its embryonic cell lineage or if its development converges to a *C. elegans* like polyclonal development.

Would it be useful to establish and compare well-chosen partial lineages of many species as a compromise between working effort and extraction of information? If yes, which parts of the cell lineage would be useful? Therefore, one

needs to identify a part of the lineage that has considerable variation between species. This comparative study shows only variation in fate specification in individual cells at the end of the cell lineage and this variation is spread over the whole cell lineage. An alternative would be to choose certain cells throughout the lineage. This method has proved its usefulness to screen a large amount of *C. elegans* mutants on lineage defects (Moskowitz et al., 1994; Hutter and Schnabel, 1994, 1995a,b). But I do not think that this method is the good one for studying the lineage of other nematode species. From this study one can see that although there is a high lineage similarity, the fate transformations between species are spread throughout the lineage. Subsampling the cell lineage would erode the data too much in order to get an idea of the similarities between species.

SUMMARY

The development of nematodes is since long considered as an autonomous, mosaic development, where developmental potential is internally determined by differential segregation of the intrinsic determinants over the different blastomeres during cell cleavage. Most of the knowledge of the development of nematodes is mainly based on the model organism *Caenorhabditis elegans*. This nematode has a strict invariant cell lineage that generates a fixed number of cells with a fixed cell type (Sulston and Horvitz, 1977; Sulston et al., 1983).

To examine the extent of conservation of this mode of development in nematodes, we established the nearly complete embryonic cell lineages of three more distantly related nematodes, *Pellioditis marina* (Rhabditidae, clade V), *Rhabditophanes* sp. (Alloionematidae, clade IV) and *Halicephalobus gingivalis* (Panagrolaimidae, clade IV). Of these three species, we recorded their embryonic development from zygote to comma-stage embryo with a 4D microscope and followed each individual cell in time and space and established the complete division pattern or cell lineage by identifying all cell divisions. This gives us a unique data set of four embryonic cell lineages, which allows us a detailed comparison between these cell lineages on the level of each individual cell.

The early development of *P. marina*, *Rhabditophanes* sp. and *H. gingivalis* start with a similar series of unequal, asynchronous cell divisions. Only the order in the sequence of these divisions differs between the three species. In early development of *H. gingivalis,* there is *a* variation in the cleavage polarity in the germline in comparison with the other species studied here. In all species studied here, the founder cells form bilateral symmetric groups in their first or second division round, except for ABa where the bilateral symmetry in the lineage is completely lost. The bilateral symmetry in the embryo of these species is secondarily restored by non-symmetric cell lineages. There are differences the inward migration of the endodermal precursors during gastrulation. In *P. marina* two E cells migrate inwards like in *C. elegans*, while this migration is delayed to the late 2E stage in *H. gingivalis* and the 4E stage in *Rhabditophanes* sp..

The topology of the nearly complete cell lineages in the species studied here is strongly conserved. 92%-95% of the determined terminal cells have the same lineage history and 78%-88% of these equivalent cells have the same cell fate. Comparison of the tissue configuration of these species shows a variable degree of conservation. The intestine, the primordial gonad and the body muscles are highly conserved in the four species, while the pharynx, the epidermis and the nervous system have a more variable configuration. The intestinal lineage of *P. marina* and *Rhabditophanes* sp. is identical to the one of *C. elegans*. *H. gingivalis* has a different E-lineage in which 18 cells and two cell deaths are formed and the establishment of the bilateral symmetry is delayed. This pattern is correlated with the concerted inward migration of the intestinal precursors with mesodermal precursors. From a mapping of these two patterns on a phylogenetic tree, one can conclude that the *C. elegans* pattern is the ancestral pattern and that the *H. gingivalis* pattern is probably derived from it. The primordial gonad is identical in all species, except for *Rhabditophanes* sp., where the germline precursors undergo an extra division to form four germline precursors instead of two in the other species. Also the body muscles are strongly conserved in number (81-83 cells) and configuration between *P. marina, C. elegans* and *Rhabditophanes* sp., although the configuration in *H. gingivalis* could not be determined. The pharynx, epidermis and nervous system are more variable tissues, with similar basic patterns but differences in individual cells.

The variation in reversal of polarity found in *H. gingivalis* is probably a derived feature that evolved from the ancestral *'C. elegans'* like pattern, which are also found in the more basal clades of the phylum (Malakhov, 1994). Then, other variations in the cleavage polarity of the germline, found in other clades must be derived too, including *A. nanus,* which has a more regulative development. If these variations in cleavage polarity are indeed derived features, then this regulative early development must be also a derived character that evolved from a non-regulative ancestor. The differences in division sequence are mainly caused by differential division rate of the germline relative to the somatic cells and may be coupled to the speed of development as proposed by Skiba and Schierenberg (1992), although this correlation is not completely clear in the species studied here. The specific patterns in the speed of the early development and the length of

the cell cycles are probably caused by the differential availability of maternal vs. zygotic material as proposed by Laugsch and Schierenberg (2004).

The differences in the intestinal patterns between *H. gingivalis* and *C. elegans* is correlated with the establishment of the bilateral symmetry in the intestine. In *H. gingivalis,* the establishment of the bilateral symmetry may be constrained by the positions of the mesodermal precursors during gastrulation. However the modification of the intestinal pattern leads to the same result, a bilateral symmetric intestine of nine rings. The concerted inward migration and close connection of the germline precursors and the intestinal cells is strongly conserved in all studied species and is found also outside the phylum in Arthropods and Vertebrates.

Based on this comparison of four nearly complete embryonic cell lineages, we conclude that there is a conservation of a '*C. elegans*' like polyclonal cell lineage with strong left-right asymmetry in clade IV and V of the phylum Nematoda. This developmental strategy is very robust, despite the large evolutionary distance and the great genomic diversity in these clades (Parkinson et al., 2004; Makedonka et al., 2005). The variations in the polyclonal fate distribution in AB are found within a conserved pattern. This pattern in *C. elegans* is strictly regulated by cell-cell inductions, which is probably conserved in clade IV and V, although more experiments should confirm this hypothesis. An evolutionary advantage of polyclonal lineages over monoclonal cell lineages is that in polyclonal cell lineages, cells are made close to the place where they are needed, thereby minimizing the need for migrations and probably speed up development. Moreover, computational modelling revealed that these polyclonal cell lineages are not as complex as they look like and that they are almost as simple as they can be, assuming constraints on the spatial positions of the cells. The strong conservation of this polyclonal pattern between species of clade IV and V suggest that this strategy has evolved before the diversification of these clades and played only a minor role in the evolution of faster species in these clades. This strategy has probably evolved from a slower, more regulative non-determined development where cells are determined 'en bloc' later in development, as can be found in the basal clade I-II.

SAMENVATTING

De ontwikkeling van nematoden wordt al lang beschouwd als een schoolvoorbeeld van mosaïsche ontwikkeling, waarbij het ontwikkelingspotentiaal van elke cel intern wordt gedetermineerd door differentiële verdeling van determinanten over de verschillende cellen. De meeste kennis over de ontwikkeling van nematoden is echter gebaseerd op het model organisme *Caenorhabditis elegans.* Deze nematode heeft een snelle ontwikkeling met een strikt invariante celgenealogie dat een vast aantal cellen met een vast celtype genereert.

Om na te gaan in hoeverre deze vorm van ontwikkeling is geconserveerd bij nematoden, wordt de bijna volledige embryonale celgenealogie opgesteld van drie nematodensoorten die ver verwant zijn met *C. elegans*: *Pellioditis marina* (Rhabditidae, clade V), *Rhabditophanes* sp. (Alloionematidae, clade IV) and *Halicephalobus gingivalis* (Panagrolaimidae, clade IV). Van deze soorten wordt de embryonale ontwikkeling in drie dimensies gefilmd met een 4D-microscoop. Zo wordt elke cel in ruimte en tijd gevolgd en wordt het volledige delingspatroon of celgenealogie opgesteld. Deze unieke dataset van vier embryonale celgenealogieën, laat een gedetailleerde vergelijking op individueel celniveau tussen deze soorten toe.

De vroege ontwikkeling van *P. marina*, *Rhabditophanes* sp. and *H. gingivalis* start met een gelijke reeks ongelijke asynchrone delingen. Enkel de volgorde waarin die delingen gebeuren, varieert tussen de drie soorten. In de vroege ontwikkeling van *H. gingivalis* is er een variatie in de delingspolariteit van de germinale lijn in vergelijking met de andere bestudeerde soorten. In alle bestudeerde soorten, vormen de stamcellen bilateraal symmetrische groepen vanaf hun eerste of tweede deling. Enkel in ABa gaat de bilaterale symmetrie in de celgenealogie volledig verloren. Desalniettemin wordt in deze soorten de bilaterale symmetrie secundair hersteld door non-symmetrische celgenealogieën. Verder zijn er verschillen in de inwaardse migratie van de endodermale precursoren tijdens de gastrulatie. In *P. marina,* gaan de twee E cellen naar binnen migreren, zoals in *C. elegans*. Deze inwaartse migratie wordt uitgesteld tot het late 2-E stadium in *H. gingivalis* en het 4E stadium in *Rhabditophanes* sp.

De topologie van de bijna volledige celgenealogieën van de bestudeerde soorten is sterk geconserveerd. 92%-95% van de gedetermineerde terminale cellen hebben dezelfde delingsgeschiedenis en 78%-88% van deze cellen vormen ook hetzelfde delingstype. Vergelijking tussen de configuratie van de weefsels toont aan dat die variabel geconserveerd is. De darm, de primordiale gonaden en de lichaamsspieren zijn sterk geconserveerd, terwijl de pharynx, de epidermis en het zenuwstelsel een meer variabele configuratie hebben. De genealogie van de darmcellen in *P. marina* en *Rhabditophanes* sp. zijn bijna identiek aan die van *C. elegans*, terwijl die van *H. gingivalis* een ander delingspatroon hebben. Dit patroon is gecorreleerd met de gezamelijke inwaartse migratie met mesodermale precursoren. Door het in kaart brengen van die twee patronen op de fylogenie, stelt men vast dat het *C. elegans* patroon ancestraal is en dat het *H. gingivalis* patroon ervan afgeleid is. De primordiale gonaden zijn identiek in alle bestudeerde soorten, met uitzondering van *Rhabditophanes* sp. waar de germinale precursoren een extra deling ondergaan en vier germinale precursorcellen vormt. Ook de lichaamsspieren hebben een sterk geconserveerd aantal cellen (81-83) en configuratie, alhoewel configuratie in *H. gingivalis* niet bepaald kon worden. De farynx, epidermis en het zenuwstelsel zijn meer variabele weefsels met een gelijkaardig basispatroon maar met individuele verschillen tussen de soorten.

De variatie in delingspolariteit van de geminale lijn in *H. gingivalis* is waarschijnlijk een afgeleid kenmerk ontstaan uit een ancestraal 'C. *elegans*' achtig patroon, dat ook wordt gevonden in de basale clades van de nematoden (Malakhov, 1994). Dan zijn ook de variaties in andere voorheen beschreven soorten, waaronder *Acrobeloides nanus,* eveneens afgeleide kenmerken. Als deze hypothese klopt, dan moet de vroege regulatieve ontwikkeling van *A. nanus* ook afgeleid zijn en onstaan zijn uit een niet regulatieve voorouder. De verschillen in de delingssequentie worden voornamelijk veroorzaakt door de differentiële delingssnelheid van de germinale lijn ten opzichte van de somatische lijn en is waarschijnlijk gecorreleerd met de snelheid van de vroege ontwikkeling, zoals gepostuleerd wordt door Skiba en Schierenberg (1992), alhoewel deze correlatie niet zo rechtlijnig is in de hier bestudeerde soorten. De specifieke patronen in de lengte van de vroege celcycli en de snelheid van vroege ontwikkeling zijn

waarschijnlijk gekoppeld aan de differentiële beschikbaarheid van maternaal vs. zygotisch materiaal zoals voorgesteld door Laugsch en Schierenberg (2004).

De verschillen in darmpatronen van *H. gingivalis* en *C. elegans* zijn gecorreleerd met het vastleggen van de bilaterale symmetrie in de primordiale darm. In *H. gingivalis* is deze uitgesteld en waarschijnlijk belemmerd door de positie van de mesodermale precursoren tijdens de gastrulatie. Niettemin leidt deze aanpassing van het darmpatroon tot hetzelfde resultaat: een bilateraal symmetrische darm bestaande uit negen opeenvolgende ringen. De gezamelijke inwaartse migratie en het nauw contact tussen de darm en de germinale precursoren is sterk geconserveerd in alle bestudeerde soorten. Dit is een algemeen thema, ook buiten de nematoden, namelijk in Arthropoda en Vertebrata.

Gebaseerd op de vegelijking van vier bijna volledige celgenealogieën, kan men besluiten dat er een sterke conservatie is van een '*C. elegans*'-achtige polyclonale celgenealogie met sterke bilaterale asymmetrie in clade IV en V van het phylum Nematoda. Deze ontwikkelingsstrategie is heel robust, ondanks de grote genetische variatie in deze clades. De variaties in het polyclonale patroon van celtypes in AB vallen binnen een geconserveerd patroon. Dit patroon wordt in *C. elegans* sterk geconserveerd door cel-cel interacties, die waarschijnlijk geconserveerd zijn in clade IV en V, hoewel meer experimentele data nodig zijn om deze hypothese te bevestigen. Een evolutionair voordeel van polyclonale celgenealogieën ten opzichte van monoclonale celgenealogieën is dat in polyclonale celgenealogieën celtypes niet worden gespecifieerd volgens positie in de genealogie maar volgens de positie in het embryo, waardoor migraties geminimaliseerd worden en waarschijnlijk een snellere ontwikkeling toelaten. Meer nog, computersimulaties hebben uitgewezen dat deze polyclonale celgenealogieën niet zo complex zijn als ze lijken en dat ze bijna zo eenvoudig mogelijk zijn als er beperkingen op de positie van cellen wordt verondersteld. Op basis van de sterke conservatie van dit polyclonale patroon bij soorten van clade IV en V, kan verondersteld worden dat deze strategie reeds voor de diversificatie van deze clades is ontstaan en dat deze strategie slechts een kleine rol heeft gespeeld in de evolutie van snellere soorten binnen deze clades. Deze strategie is waarschijnlijk geëvolueerd uit een tragere, regulatieve, niet-gedetermineerde ontwikkeling, zoals die voorkomt in de basale clades I-II.

LIST OF PUBLICATIONS

This PhD project is published in:

Houthoofd, W., Jacobsen, K., Mertens, C., Vangestel, S., Coomans, A. and Borgonie, G. (2003). Embryonic cell lineage of the marine nematode *Pellioditis marina*. *Developmental Biology* 258, 57-69.

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Houthoofd, W., Willems, M., Coomans, A. and Borgonie, G. Conserved embryonic cell lineages in genetically diverse nematodes. in prep.

Houthoofd, W., Willems, M., and Borgonie, G. The embryonic cell lineage of *Rhabditophanes* sp. in prep.

Houthoofd, W. Schnabel, R. and Borgonie, G. The embryonic cell lineage of *Halicephalobus gingivalis*. in prep.

Houthoofd, W., Artois, T., Vangestel, S., Willems, M., Mertens, C. and Borgonie, G. Phylogenetic analysis of embryonic cell lineage data, with an example from Nematoda. in prep.

Other publications

Bert, W., Messiaen M., Manhout J., Houthoofd W. and Borgonie, G. (2006). Evolutionary loss of parasitism by nematodes? Discovery of a free-living filarioid nematode. *Journal of Parasitology*, in press.

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CHAPTER 1: INTRODUCTION

Figure 1.1: Molecular phylogeny of the phylum Nematoda.

based on 18S rDNA (Blaxter et al., 1998).

CHAPTER 3: EARLY DEVELOPMENT

Table 3.1: Division sequence of the first divisions

Comparison of the division sequence of the early division of the founder cells between *C. elegans* (Ce), *Rhabditophanes* sp. (Rh), *P. marina* (Pm) and *H. gingivalis* (Hg). The germline cells are marked in red. Divisions that occur simultaneously or vary within the same species are grouped in a red square. The relative early developmental tempo was measured from the first division of AB and the division of E and normalised with the tempo of *C. elegans*.

Sequence of cell divisions		*Ce*	*Rh*	*P m*	*Hg*
	1	**P_0**	**P_0**	**P_0**	**P_0**
	2	AB	AB	AB	AB/ **P_1**
	3	**P_1**	**P_1**	**P_1**	
	4	2AB	2AB/EMS/	2AB	**P_2**
	5	EMS	**P_2**	EMS	2AB
	6	**P_2**		**P_2**	EMS
	7	4AB	4AB/E/MS	4AB	4AB
	8	MS		MS	MS
	9	E		E	**P_3**
	10	C	**P_3**	**P_3**	8AB
	11	**P3**/8AB	C	C	E
P_4 present		24	15	15	14
relative early developmental tempo		1,0	1,2	2,5	2,9
relative tempo until muscle contraction		1.0	1.1	1.7	1,8
# specimens		10	3	3	3

Table 3.2: Secondary symmetries in the AB-lineage

List of non-symmetrical sublineage pairs that give rise to symmetrical cells in *C. elegans* divided in two classes: **A.** Left and right lineages that give rise to symmetrical cells; **B.** Lineages from the same side that give rise to symmetrical cells. In the last column is indicated in which species each secondary symmetry is present. *C. elegans* (C), *P. marina* (P), *Rhabditophanes* sp. (R) and *H. gingivalis* (H).

left	right	
A. Left and right lineages that give rise to symmetrical cells		
AB alp aaa	AB ara paa	CH
AB alp aap	AB ara aap	CPRH
AB alp apa	AB ara ppa	CRH
AB alp app	AB ara pap	CRH
AB alp pap	AB ara ppp	CRH
AB alp pp	AB pra aa	CPR
AB pla aa	AB arp ap	CPRH
B. Lineages from the same side that give rise to symmetrical cells.		
AB ala aaa a	AB ala apa a	CPH
AB ala aap a	AB ala aap p	CPRH
AB ala app p	AB ala paa p	CPRH
AB ala paa a	AB ala ppa p	CPRH
AB ala pap	AB ala ppp	CPRH
AB alp paa a	AB ala app a	CPRH
AB alp paa p	AB ala apa p	CR
AB ara aaa aa	AB ara aaa ap	CPR
AB ara apa	AB ara app	CPRH
AB arp aaa ap	AB arp aaa pa	CPR
AB arp pa	AB arp pp	CPRH

Figure 3.1: Cell lineages of the first divisions

The early cell lineages and the formation of the founder cells (somatic cells AB, MS, E, C, D and germline precursor P_4). Vertical axis represents time. Timeline in minutes after first division of AB. The scale of the timeline is equal in all four cell lineages. Note that the developmental speed and the division sequence is different in the four species. Left branch is anterior daughter, right branch posterior daughter.

Figure 3.2: DIC images of the first divisions in *P. marina*

All stages anterior to the left, scalebar is 5µm. **A.** 4-cell stage,left lateral view, just after division of AB and P_1. **B.** 8-cell stage, right-ventral view. EMS has divided in an anterior MS cell and a posterior E cell. P_3 cell lies behind E. **C.** 16-cell stage, ventral view.MS has divided in an anterior MSa and a posterior MSp and E has divided in anterior Ea and posterior Ep D and P4 lie next to each other **D.** Late comma (2-fold) stage at the onset of body muscle contraction. left lateral view.

Figure 3.3: DIC images of the first divisions in *Rhabditophanes* sp.

All stages anterior to the left, scalebar is 5µm. **A.** Early 4-cell stage,left lateral view, just after division of AB and P_1. **B.** Late four cell stage, left lateral view. Rhomboid shape. EMS lies at the future ventral side of the embryo, ABp at the future dorsal side. **C.** 8-cell stage, ventral view. EMS has divided in an anterior MS cell and a posterior E cell. P_3 cell lies behind E. **E.** 16-cell stage, ventral view.MS has divided in MSa and MSp and E has divided in anterior Ea and posterior Ep **E.** 32-cell stage, ventral view. The four E cells lie at the ventral side just before gastrulation, when these two cells move inwards. **F.** Late comma (2-fold) stage at the onset of body muscle contraction. right lateral view. Arrowheads delineate the outline of the pharynx.

Figure 3.4: DIC images of the first divisions in *H. gingivalis*

All stages anterior to the left, scalebar is 5µm. **A.** two cell stage. **B.** Early 4-cell stage,left lateral view, just after division of AB and P_1. Note the perpendicular division axes of the two cells, which results in a T-shaped embryo. **C.** Late four cell stage, left lateral view. Rhomboid shape. EMS lies at the future ventral side of the embryo, ABp at the future dorsal side. **D.** 8-cell stage, left lateral view. EMS has divided in an anterior MS cell and a posterior E cell. P_2 has divided into an anterior P_3 cell and a posterior C cell. Due to constriction of space by the eggshell, C is pushed to the dorsal side. **E.** 14-cell stage, left ventral view. P_3 has divided in an anterior D cell and a posterior P_4 cell, also here the posterior P_4 cell is pushed to the dorsal side. **F.** 26-cell stage, ventral view. The two E cells lie at the ventral side just before gastrulation, when these two cells move inwards. **G**. Early comma stage, during morphogenesis (elongation of the body). **H.** Late comma (2-fold) stage at the onset of body muscle contraction.

Figure 3.5: Number of cells during embryonic development

C. elegans (black), *Rhabditophanes* sp. (blue), *P. marina* (green) and *H. gingivalis* (red). Time in the X-axis is normalised from the first division of AB. The cell count of *Rhabditophanes* sp. and *H. gingivalis* stop prematurely since the precise cell number could not be determined due to the unresolved cells and mitoses at the end of the recordings.

Figure 3.6: Cell cycle length of AB generations.

The median length of AB cells in each generation is plotted in time. *C. elegans* (black), *Rhabditophanes* sp. (blue), *P. marina* (green) and *H. gingivalis* (red).

Figure 3.7 Cell division periods per founder cell

Comparison of cell divisions rounds in time (min after division of AB) between *C. elegans* (black), *Rhabditophanes* sp. (blue), *P. marina* (green) and *H. gingivalis* (red).. On each horizontal line the cell division events of one founder cell are given. The order from top to bottom is by decreasing length of cell cycle. Each box indicates the time from the division of the first cell to the division of the last cell of a lineage. The division of each founder cell is indicated with its name. The number between each pair of boxes of each dvision round indicates the number of cells of that divide in this generation.

Figure 3.8: Bilateral symmetry of the founder cells

The bilateral symmetry in the *P. marina* (A) and *C. elegans* (B) embryo. Dorsal view, anterior left. Left branches are the anterior daughters of divisions; right branches are the posterior daughters. Differences occur in Caa and Cpa and anteriorly in ABar and ABal. a, anterior; p, posterior; l, left; r, right.

Figure 3.9: Symmetry breakage in C lineage in *P. marina*

Correction of bilateral symmetry breakage in the body muscle precursors Cap (red) and Cpp (yellow) in *P. marina* (A, B) compared with *C. elegans* (C, D). Ventral view, anterior to the left. A. 240 minutes (90-cell stage); B. 300 minutes (171-cell stage); C. 123 minutes (87-cell stage); D. 160 minutes (167-cell stage).

Figure 3.10: Secondary symmetries in AB

Lineal relationships of the 21 pairs of contralaterally symmetric cells in the AB lineage of *C. elegans* (see alsoTable 3.2). The lineage is oriented with the anterior cells at the top of the figure and the left cells at the left side of the figure. Pairs of cells shown at each side of the midline are bilateral symmetric. The members of each pair occupy approximately equivalent positions on the left and right sides of the embryo. Note that the bilateral symmetry between symmetric lineages in ABa is completely lost. Equivalent cell lineages (eg. ABala and ABare) form different cells at each side of the embryo. The lineages on the left (ABala and ABalp) are shifted towards the anterior. This is caused by the skewed left-right division of ABa and ABp (marked in big circles) into respectively ABal, ABar and ABpl, ABpr (marked in small circles).

Figure 3.11: 3D reconstructions of secondary symmetries in AB

3D-reconstructions of *H. gingivalis* embryo, illustrating loss and recovery of bilateral symmetry in AB lineage. Every ball represents the position of the nucleus of each cell. Cells on the left side are shown in dark colors; cells on the right side are shown in light colors **A.** 4AB stage (8-cell stage). Dorsal view, anterior up. After the left-right division of ABa and ABp, the left daughters ABal (dark blue) and ABpl (dark green) are skewed to the anterior in relation to their right daughters ABar (light blue) and ABpr (light green) **B.** 8AB stage (14-cell stage). Dorsal view, anterior up. After the next division, the bilateral symmetry of the anterior AB granddaughters is lost, while the symmetry of the posterior granddaughters is largely retained. ABala: dark blue, ABalp: dark purple; ABara: light blue; ABarp: light purple. ABpla: dark green; ABplp: dark yellow; ABpra: light green, ABprp: light yellow. MS: grey; E: brown; C; red; D; green blue; P_4: black. **C-E.** 256AB stage (360 cell stage). C**.** Anterior view, dorsal side up**.** Anterior secondary bilateral symmetries. ABalaaaaa (darkblue) vs ABalaapaa (light blue). ABalaaapa (dark green) vs ABalaaapp (light green). ABalapaap (dark purple) vs ABalaappp (light purple) ABalapaaa (red) vs. ABalappap (orange); ABalappa (dark yellow) vs. ABalappp (light yellow). ABalppaaaa (black) vs. ABalaappa (grey) **D.** Dorsal view, anterior up. ABarpaaaap (darkblue) vs ABarpaaapa (light blue). ABplaaa (dark green) vs ABarpap (light green). ABarppa (dark purple) vs ABarppp (light purple) ABaraapa (red) vs. ABaraapp (orange); **E.** Ventral view, anterior up. ABalpaaa (darkblue) vs ABarapaa (light blue). ABalpaap (dark green) vs ABaraaap (light green). ABalpapa (dark purple) vs ABarappa (light purple) ABalpapp (red) vs. ABarapap (orange); ABalppap (dark yellow) vs. ABarappp (light yellow).

Movie 3.1: gastrulation in *P. marina*

Time lapse movie of the inward migation of the intestinal precursors during gastrulation. Based on subsequent 3D reconstructions; balls represent the positions of the nucleus of each cell. Right lateral view, ventral side at the top. MSa, dark grey; MSp light grey; E, brown; P_4 dark blue, D light blue. Time is marked at right up corner in minutes after division of AB.

Movie 3.2: gastrulation in *Rhabditophanes* sp.

Time lapse movie of the inward migation of the intestinal precursors during gastrulation. Based on subsequent 3D reconstructions; balls represent the positions of the nucleus of each cell. Right lateral view, ventral side at the top. MSa, dark grey; MSp light grey; E, brown; P_4 dark blue, D light blue. Time is marked at right up corner in minutes after division of AB.

Movie 3.3: gastrulation in *H. gingivialis*

Time lapse movie of the inward migation of the intestinal precursors during gastrulation. Based on subsequent 3D reconstructions; balls represent the positions of the nucleus of each cell. Right lateral view, ventral side at the top. MSa, dark grey; MSp light grey; E, brown; P_4 dark blue, D light blue. Time is marked at right up corner in minutes after division of AB.

CHAPTER 4: ORGANOGENESIS

Table 4.1: Comparative cell list of terminal cells with their cell fate.

Comparison between *Rhabditophanes* sp. (Rh), *H. gingivalis* (Hg), *P. marina* (Pm) and *C. elegans* (Ce). AB cells are grouped per great-granddaughter of AB and in the names of the cells saces are left between groups of three letters to improve readability. The fate of each cell is marked with a letter and color code in accordance with the color code of the cell lineage figures. blue: neuron (N); green: pharynx (P); purple: epidermis (E); darkblue: other fate (O); grey: programmed cell death (D); orange: body muscle (U); dark green: intestine (I); gonad (G); unresolved cell (F); unresolved mitosis (M?); mitosis (M).

		Rh		*Hg*		*Pm*	*Ce*
	name	fate	name	fate	name	fate	fate
AB ala	aaa lal	N	aaa lal	N	aaa lal	N	N
	aaa lar	N	aaa lar	F	aaa lar	N	**D**
	aaa lpa	N	aaa lpa	N	aaa lpa	N	**D**
	aaa lpp	N	aaa lpp	F	aaa lpp	N	N
	aaa rla	N	aaa rla	N	aaa rla	N	**D**
	aaa rlp	N	aaa rlp	N	aaa rlp	N	N
	aaa rra	N	aaa rra	N	aaa rra	N	**D**
	aaa rrp	N	aaa rrp	N	aaa rrp	N	N
	aap all	N	aap all	N	aap all	N	N
	aap alr	N	aap alr	N	aap alr	N	N
	aap arl	N	aap arl	F	aap arl	P	**D**
	aap arr	N	aap arr	N	aap arr	N	N
	aap pll	N	aap pll	N	aap pll	N	N
	aap plr	N	aap plr	N	aap plr	**D**	**D**
	aap prl	N	aap pr	M?	aap prl	N	N
	aap prr	N			aap prr	N	N
	apa aal	N	apa aal	**D**	apa aal	**D**	**D**
	apa aar	N	apa aar	F	apa aar	N	N
	apa apa	N	apa apa	F	apa apa	N	**D**
	apa app	N	apa app	N	apa app	N	N
	apa pa	F	apa pa	F	apa pa	**D**	**D**
	apa ppa	N	apa ppa	F	apa ppa	N	**D**
	apa ppp	N	apa ppp	N	apa ppp	N	N
	app aa	**D**	app aa	**D**	app aa	**D**	**D**
	app apa	N	app apa	N	app apa	N	N
	app app	N	app app	N	app app	N	N
	app paa	N	app paa	F	app paa	N	N
	app pap	N	app pap	N	app pap	**D**	**D**
	app pp	M?	app ppa	F	app ppa a	N	N
					app ppa p	**D**	**D**
			app ppp	F	app ppp	N	N
	paa aaa	N	paa aa	M?	paa aaa	N	N
	paa aap	N			paa aap	N	**D**
	paa apa	N	paa apa	N	paa apa	N	N
	paa app	N	paa app	N	paa app	N	N
	paa paa	N	paa paa	N	paa paa	E	N
	paa pap	N	paa pap	N	paa pap	**D**	**D**
	paa ppa	N	paa ppa	F	paa ppa a	N	N
					paa ppa p	**D**	**D**
	paa ppp	N	paa ppp	N	paa ppp	N	N
	pap aaa	N	pap aa	F	pap aa	**D**	**D**
	pap aap	N					
	pap apa	N	pap apa	N	pap apaa	N	N
					pap apap	N	**D**
	pap app	N	pap app	N	pap app	N	N
	pap paa	N	pap paa	N	pap paaa	N	N
					pap paap	**D**	**D**
	pap pap	N	pap pap	F	pap pap	**D**	N
	pap ppa	N	pap ppa	N	pap ppa	N	N
	pap ppp	N	pap ppp	N	pap ppp	N	N
	ppa aa	**D**	ppa aa	F	ppa aa	**D**	**D**

	ppa apa	N	ppa ap	M?	ppa apa	N	N
	ppa app	N			ppa app	**D**	**D**
	ppa paa	N	ppa paa	N	ppa paa	N	N
	ppa pap	N	ppa pap	N	ppa pap	**D**	**D**
	ppa ppa	N	ppa ppa	N	ppa ppa	N	N
	ppa ppp	N	ppa ppp	N	ppa ppp	N	N
	ppp aaa	N	ppp aaa	**D**	ppp aaa	P	N
	ppp aap	N	ppp aap	N	ppp aap	P	N
	ppp apa	N	ppp apa	N	ppp apaa	N	N
					ppp apap	**D**	**D**
	ppp app	N	ppp app	N	ppp app	N	N
	ppp pa	M?	ppp paa	N	ppp paaa	N	N
					ppp paap	**D**	**D**
			ppp pap	N	ppp pap	N	N
	ppp ppa	N	ppp ppa	N	ppp ppa	N	N
	ppp ppp	N	ppp ppp	N	ppp ppp	N	N
AB alp	aaa aaa	P	aaa aaa	P	aaa aaa	P	P
	aaa aap	P	aaa aap	P	aaa aap	P	P
	aaa apa	P	aaa apa	P	aaa apa	P	P
	aaa app	P	aaa app	P	aaa app	**D**	**D**
	aaa paa	P	aaa paa	P	aaa paa	P	P
	aaa pap	P	aaa pap	P	aaa pap	**D**	**D**
	aaa ppa	P	aaa ppa	P	aaa ppa	**D**	**D**
	aaa ppp	P	aaa ppp	P	aaa ppp	E	P
	aap aaa	P	aap aaa	P	aap aaa	E	P
	aap aap	P	aap aap	P	aap aap	E	P
	aap apa	P	aap apa	P	aap apa	P	P
	aap app	P	aap app	P	aap app	P	P
	aap paa	P	aap paa	P	aap paa	N	P
	aap pap	P	aap pap	P	aap pap	N	P
	aap ppa	E	aap ppa	E	aap ppa	E	E
	aap ppp	E	aap ppp	E	aap ppp	E	E
	apa aaa	N	apa aaa	N	apa aaa	N	N
	apa aap	E	apa aap	E	apa aap	N	E
	apa apa	N	apa apa	N	apa apa	N	P
	apa app	F	apa app	**D**	apa app	**D**	**D**
	apa paa	P	apa paa	N	apa paa	P	N
	apa pap	F	apa pap	N	apa pap	P	M
	apa ppa	P	apa ppa	**D**	apa ppa	**D**	**D**
	apa ppp	N	apa ppp	N	apa ppp	N	N
	app aaa	F	app aaa	**D**	app aaa	**D**	**D**
	app aap	P	app aap	N	app aap	N	M
	app apa	P	app apa	P	app apa	N	P
	app app	P	app app	P	app app	P	P
		P	app pa	F	app paa	N	P
	app pap	**D**			app pap	**D**	**D**
	app ppa	P	app ppa	P	app ppa	N	P
	app ppp	P	app ppp	P	app ppp	N	P
	paa aa	**D**	paa aa	**D**	paa aa	**D**	**D**
	paa apa	N	paa apa	N	paa apa	P	N
	paa app	N	paa app	N	paa app	P	N
	paa paa	N	paa pa	**D**	paa pa	**D**	**D**
	paa pap	N					
	paa ppa	N	paa ppa	N	paa ppa	P	**D**
	paa ppp	F	paa ppp	N	paa ppp	P	N

	pap aaa	N	pap aaa	F	pap aaa	P	N
	pap aap	N	pap aap	N	pap aap	P	N
	pap apa	N	pap apa	N	pap apa	P	N
	pap app	N	pap app	N	pap app	P	N
	pap paa	N	pap paa	N	pap paa	N	N
	pap pap	N	pap pap	N	pap pap	N	N
	pap ppa	N	*pap ppa*	N	*pap ppa*	N	M
	pap ppp	N	pap ppp	N	pap ppp	N	N
	ppa aaa	N	ppa aaa	N	ppa aaa	N	N
	ppa aap	N	ppa aap	N	ppa aap	N	N
	ppa apd	N	ppa apd	N	ppa apd	N	N
	ppa apv	F	ppa apv	F	ppa apv	P	**D**
	ppa pad	N	ppa pad	N	ppa pad	E	N
	ppa pav	N	ppa pav	N	ppa pav	E	N
	ppa ppa	N	ppa ppa	F	ppa ppa	E	**D**
	ppa ppp	N	ppa ppp	F	ppa ppp	**D**	M
	ppp aa	M?	ppp aad	N	ppp aad	N	N
			ppp aav	**D**	ppp aav	**D**	**D**
	ppp apa	N	ppp apa	N	ppp apa	P	M
	ppp app	N	ppp app	**D**	ppp app	**D**	**D**
	ppp paa	N	ppp paa	N	ppp paa	P	N
	ppp pap	N	ppp pap	N	ppp pap	P	N
	ppp ppa	N	ppp ppa	N	ppp ppaa	**D**	N
					ppp ppap	P	**D**
	ppp ppp	N	ppp ppp	N	ppp pppa	P	N
					ppp pppp	P	N
AB ara	aaa aaa	P	aaa aaa	P	aaa aaa	P	P
	aaa aap	P	aaa aap	P	aaa aap	P	P
	aaa apa	P	aaa apa	P	aaa apa	P	P
	aaa app	P	aaa app	P	aaa app	P	P
	aaa paa	F	aaa paa	P	aaa paa	P	**D**
	aaa pap	P	aaa pap	P	aaa pap	P	P
	aaa pp	**D**	aaa pp	**D**	aaa pp	**D**	**D**
	aap aaa	P	aap aaa	P	aap aaa	P	P
	aap aap	P	aap aap	P	aap aap	P	P
	aap apa	P	aap apa	P	aap apa	N	P
	aap app	P	aap app	P	aap app	P	P
	aap paa	P	aap paa	P	aap paa	P	P
	aap pap	P	aap pap	P	aap pap	N	P
	aap ppa	P	aap ppa	P	aap ppa	N	P
	aap ppp	F	aap ppp	P	aap ppp	N	E
	apa aaa	P	apa aaa	P	apa aaa	P	P
	apa aap	P	apa aap	P	apa aap	P	P
	apa apa	P	apa apa	P	apa apa	P	P
	apa app	P	apa app	P	apa app	P	P
	apa paa	P	apa paa	P	apa paa d	P	**D**
					apa paa v	P	P
	apa pap	P	apa pap	P	apa pap	P	P
	apa ppa	P	apa ppa	P	apa ppa	P	P
	apa ppp	P	apa ppp	F	apa ppp	P	P
	app aaa	P	app aaa	P	app aaa	N	P
	app aap	P	app aap	P	app aap	P	P
	app apa	P	app apa	P	app apa	N	P
	app app	P	app app	P	app app	P	P
	app paa	P	app paa	P	app paa d	P	**D**

					app paa v	P	P
	app pap	P	app pap	P	app pap	P	P
	app ppa	P	app ppa	P	app ppa	N	P
	app ppp	P	app ppp	P	app ppp	P	P
	paa aaa	P	paa aaa	P	paa aaa	P	P
	paa aap	F	paa aap	P	paa aap	P	P
	paa apa	P	paa apa	P	paa apa	P	P
	paa app	P	paa app	P	paa app	**D**	**D**
	paa paa	P	paa paa	P	paa paa	P	P
	paa pap	F	paa pap	F	paa pap	N	**D**
	paa ppa	P	paa ppa	P	paa ppa	P	P
	paa ppp	P	paa ppp	P	paa ppp	P	P
	pap aaa	**D**	pap aaa	**D**	pap aaa	**D**	**D**
	pap aap	P	pap aap	P	pap aap	P	M
	pap apa	F	pap apa	P	pap apa	P	P
	pap app	N	pap app	F	pap app	P	P
	pap paa	P	pap paa	P	pap paa	N	P
	pap pap	P	pap pap	P	pap pap	**D**	**D**
	pap ppa	P	pap ppa	P	pap ppa	P	P
	pap ppp	P	pap ppp	P	pap ppp	P	P
	ppa aaa	F	ppa aaa	N	ppa aaa	P	N
	ppa aap	N	ppa aap	F	ppa aap	**D**	**D**
	ppa apa	E	ppa apa	F	ppa apa	E	E
	ppa app	E	ppa app	**D**	ppa app	**D**	**D**
	ppa paa	N	ppa paa	N	ppa paa	N	N
	ppa pap	N	ppa pap	N	ppa papa	**D**	**D**
					ppa papp	N	N
	ppa ppa	N	ppa ppa	N	ppa ppa	N	N
	ppa ppp	N	ppa ppp	N	ppa ppp	N	N
	ppp aaa	P	ppp aaa	N	ppp aaa	P	N
	ppp aap	F	ppp aap	N	ppp aap	P	N
	ppp apa	F	ppp apa	N	ppp apa	N	N
	ppp app	N	ppp app	N	ppp app	N	N
	ppp paa	N	ppp paa	N	ppp paa	P	N
	ppp pap	N	ppp pap	N	ppp pap	N	N
	ppp ppa	N	ppp ppaa	N	ppp ppaa	N	N
			ppp ppap	N	ppp ppap	**D**	**D**
	ppp ppp	N	ppp ppp	N	ppp ppp	N	N
AB arp	aaa aaa	N	aaa aa	M?	aaa aaa	N	N
	aaa aap	N			aaa aap	N	N
	aaa apa	N	aaa ap	M?	aaa apa	E	N
	aaa app	E			aaa app	E	N
	aaa paa	N	aaa pa	M?	aaa paa	N	N
	aaa pap	N			aaa pap	N	N
	aaa pp	**D**	aaa pp	**D**	aaa pp	**D**	**D**
	aap aa	E	aap aa	E	aap aa	E	E
	aap ap	E	aap ap	E	aap ap	E	E
	aap pa	E	aap pa	E	aap pa	E	E
	aap pp	E	aap pp	E	aap pp	E	E
	apa aaa	N	apa aaa	N	apa aaa	N	N
	apa aap	N	apa aap	N	apa aap	N	N
	apa apa	F	apa apa	N	apa apa	**D**	**D**
	apa app	F	apa app	N	apa app a	N	N
					apa app p	N	N
	apa pa	E	apa pa	E	apa pa	E	E

	apa pp	E	apa pp	E	apa pp	E	E
	app aa	E	app aa	E	app aa	E	E
	app ap	E	app ap	E	app ap	E	E
	app pa	E	app pa	E	app pa	E	E
	app pp	E	app pp	E	app pp	E	E
	paa aa	E	paa aa	E	paa aa	E	N
	paa ap	E	paa ap	E	paa ap	E	E
	paa pa	E	paa pa	E	paa pa	E	E
	paa pp	E	paa pp	E	*paa pp*	E	M
	pap aa	E	pap aa	E	pap aa	E	E
	pap ap	E	pap ap	E	pap ap	E	E
	pap pa	E	pap pa	E	pap pa	E	E
	pap pp	E	pap pp	E	pap pp	E	E
	ppa aa	E	ppa aa	E	ppa aa	E	N
	ppa ap	E	ppa ap	E	ppa ap	E	E
	ppa pa	E	ppa pa	E	ppa pa	E	E
	ppa pp	E	ppa pp	F	ppa pp	E	M
	ppp aa	E	ppp aa	E	ppp aa	E	E
	ppp ap	E	ppp ap	E	ppp ap	E	E
	ppp pa	E	ppp pa	E	ppp pa	E	E
	ppp pp	E	ppp pp	E	ppp pp	E	E
AB pla	aaa aaa	N	aaa aa	M?	aaa aaa	N	N
	aaa aap	N			aaa aap	N	N
	aaa apa	N	aaa ap	M?	aaa apa	**D**	**D**
	aaa app	N			aaa app a	E	N
					aaa app p	N	N
	aaa pa	E	aaa pa	F	aaa pa	E	E
	aaa pp	E	aaa pp	E	aaa pp	E	E
	aap aa	E	aap aa	E	aap aa	E	E
	aap ap	E	aap ap	E	aap ap	E	E
	aap pa	E	aap pa	E	aap pa	E	E
	aap pp	E	aap pp	E	aap pp	E	E
	apa aa	M?	apa aaa	E	apa aaa	E	E
			apa aap	F	apa aap	N	N
	apa apa	N	apa apa	N	apa apa	E	N
	apa app	N	apa app	N	apa app	N	N
	apa paa	N	apa paa	N	apa paa	N	N
	apa pap	N	apa pap	N	apa pap	N	N
	apa ppa	N	apa ppa	E	apa ppa	N	N
	apa ppp	E	apa ppp	E	apa ppp	E	M
	app aa	E	app aa	E	app aa	E	E
	app ap	E	app ap	E	app ap	E	E
	app pa	E	app pa	E	app pa	E	E
	app pp	E	app pp	E	app pp	E	E
	paa aa	M?	paa aaa	N	paa aaa	**D**	**D**
			paa aap	N	paa aap	N	M
	paa apa	N	paa apa	N	paa apa	N	M
	paa app	N	paa app	F	paa app	N	N
	paa pa	E	paa pa	E	paa pa	E	E
	paa pp	E	paa pp	E	paa pp	E	E
	pap aa	E	pap aa	E	pap aa	E	M
	pap ap	E	pap ap	E	pap ap	E	E
	pap pa	E	pap pa	E	pap pa	E	E
	pap ppa	**D**	pap ppa	E	pap ppa	**D**	**D**

	pap ppp	N	pap ppp	E	pap ppp	N	M
	ppa aa	E	ppa aa	E	ppa aa	E	E
	ppa ap	E	ppa ap	E	ppa ap	E	E
	ppa pa	E	ppa pa	E	ppa pa	E	E
	ppa pp	E	ppa pp	E	ppa pp	E	E
	ppp aa	M?	ppp aa	F	*ppp aa*	E	M
			ppp apa	F	ppp apa	**D**	**D**
	ppp ap	M?	ppp app	N	ppp appa	E	N
					ppp appp	E	N
	ppp pa	E	ppp pa	E	ppp pa	E	E
	ppp pp	E	ppp pp	E	ppp pp	E	E
AB plp	aaa aaa	N	aaa aaa	N	aaa aaa	N	N
	aaa aap	N	aaa aap	N	aaa aap	N	N
	aaa apa	N	aaa apa	O	aaa apa	E	O
	aaa app	N	aaa app	N	aaa app	N	N
	aaa pa	M?	aaa paa	N	aaa paa	N	N
			aaa pap	N	aaa pap	N	N
	aaa ppa	N	aaa ppa	N	aaa ppa a	E	N
					aaa ppa p	**D**	**D**
	aaa ppp	N	aaa ppp	N	aaa ppp	P	N
	aap aa	M?	aap aaa	N	aap aaa	N	N
			aap aap	N	aap aap	N	N
	aap apa	N	aap apa	N	aap apa	N	N
	aap app	N	aap app	N	aap app	E	N
	aap paa	N	aap pa	M?	aap paa	E	N
	aap pap	N			aap pap	E	N
	aap pp	M?	aap ppa	N	aap ppa	E	N
			aap ppp	F	aap ppp	E	M
	apa aaa	N	apa aaa	N	apa aaa	N	N
	apa aap	N	apa aap	N	apa aap	N	N
	apa apa	F	apa apa	N	apa apa	P	N
	apa app	F	apa app	N	apa app	P	N
	apa paa	O	apa paa	N	apa paa	P	O
	apa pap	N	apa pap	N	apa pap	P	N
	apa ppa	N	apa ppa	N	apa ppa	P	N
	apa ppp	N	apa ppp	N	apa ppp	N	N
	app aaa	P	app aaa	N	app aaa	P	N
	app aap	O	app aap	O	app aap	E	O
	app ap	**D**	app ap	**D**	app ap	**D**	**D**
	app paa	O	app paa	O	app paa	O	O
	app pap	O	app pap	O	app pap	O	O
	app ppa	N	app ppa	O	app ppa	N	N
	app ppp	O	app ppp	O	app ppp	O	O
	paa aaa	N	paa aaa	N	paa aaa	N	N
	paa aap	N	paa aap	N	paa aap	P	M
	paa ap	**D**	paa ap	F	paa ap	**D**	**D**
	paa paa	N	paa paa	F	paa paa	**D**	**D**
	paa pap	N	paa pap	N	paa pap	E	N
	paa ppa	N	paa ppa	N	paa ppa	E	N
	paa ppp	N	paa ppp	N	paa ppp	N	N
	pap aaa	N	pap aaa	N	pap aaaa	P	N
					pap aaap	P	N
	pap aap	N	pap aap	N	pap aap	P	N
	pap apa	N	pap apa	N	pap apa	N	N

	pap app	N	pap app	N	pap app	N	N
	pap paa	N	pap paa	N	pap paa	E	N
	pap pap	N	pap pap	N	pap pap	N	N
	pap ppa	N	pap ppa	N	pap ppa	N	N
	pap ppp	N	pap ppp	N	pap ppp	N	N
	ppa aaa	N	ppa aaa	N	ppa aaa	N	N
	ppa aap	N	ppa aap	N	ppa aap	N	N
	ppa apa	N	*ppa apa*	N	*ppa apa*	E	M
	ppa app	N	ppa app	N	ppa app	E	N
	ppa paa	N	ppa paa	N	ppa paa	E	N
	ppa pap	F	ppa pap	N	ppa pap	E	E
	ppa ppa	F	ppa pp	**D**	ppa pp	**D**	**D**
	ppa ppp	F					
	ppp aaa	N	ppp aaa	O	ppp aaa	E	N
	ppp aap	O	ppp aap	O	ppp aap	O	O
	ppp apa	O	ppp apa	O	ppp apa	O	O
	ppp app	O	ppp app	O	ppp app	O	O
	ppp pa	M?	ppp paa	O	ppp paa	O	O
			ppp pap	O	ppp pap	O	O
	ppp ppa	O	ppp ppa	E	ppp ppa	O	**D**
	ppp ppp	E	ppp ppp	E	ppp ppp	O	E
AB pra	aaa aaa	N	aaa aaa	N	aaa aaa	N	N
	aaa aap	N	aaa aap	N	aaa aap	N	N
	aaa apd	N	aaa ap	M?	aaa apd	N	N
	aaa apv	F			aaa apv	**D**	**D**
	aaa pad	N	aaa pad	N	aaa pad	N	N
	aaa pav	N	aaa pav	N	aaa pav	N	N
	aaa ppa	F	aaa ppa	N	aaa ppa	**D**	**D**
	aaa ppp	F	aaa ppp	N	aaa ppp a	N	N
					aaa ppp p	**D**	**D**
	aap aaa	N	aap aaa	N	aap aaa	N	N
	aap aap	F	aap aap	F	aap aap	E	**D**
	aap apa	N	aap apa	N	aap apa	N	M
	aap app	N	aap app	N	aap app	N	**D**
	aap paa	F	aap paa	N	aap paa	N	N
	aap pap	F	aap pap	N	aap pap	N	N
	aap ppa	F	aap ppa	N	aap ppa	N	N
	aap ppp	F	aap ppp	N	aap ppp a	N	N
					aap ppp p	N	N
	apa aaa	N	apa aaa	N	apa aaa	E	E
	apa aap	N	apa aap	N	apa aap	N	N
	apa apa	N	apa apa	N	apa apa	N	N
	apa app	N	apa app	N	apa app	N	N
	apa paa	N	apa paa	N	apa paa	N	N
	apa pap	N	apa pap	N	apa pap	N	N
	apa ppa	N	apa ppa	N	apa ppa	N	N
	apa ppp	N	apa ppp	N	apa ppp a	N	N
					apa ppp p	N	**D**
	app aa	E	app aa	F	app aa	E	E
	app ap	E	app ap	E	app ap	E	E
	app pa	E	app pa	E	app pa	E	E
	app pp	E	app pp	E	app pp	E	E
	paa aaa	N	paa aa	E	paa aaa	**D**	**D**
	paa aap	N			paa aap	E	M
	paa apa	N	paa ap	E	paa apad	E	N

					paa apav	E	N
	paa app	N			paa app	E	N
	paa pa	E	paa pa	E	paa pa	E	O
	paa pp	E	paa pp	E	paa pp	E	E
	pap aa	E	pap aa	E	pap aa	E	M
	pap ap	E	pap ap	E	pap ap	E	E
	pap pa	E	pap paa	N	pap pa	E	E
			pap pap	N			
	pap pp	M?	pap ppa	N	pap ppa	**D**	**D**
			pap ppp	N	pap ppp	E	M
	ppa aa	E	ppa aa	E	ppa aa	E	E
	ppa ap	E	ppa ap	E	ppa ap	E	E
	ppa pa	E	ppa pa	E	ppa pa	E	E
	ppa pp	E	ppa pp	E	ppa pp	E	E
	ppp aaa	N	ppp aaa	N	ppp aaa	N	N
	ppp aap	F	ppp aap	N	ppp aap	**D**	**D**
	ppp ap	M?	ppp ap	E	ppp apa	**D**	**D**
					ppp app	E	M
	ppp pa	E	ppp pa	E	ppp pa	E	E
	ppp pp	E	ppp pp	E	ppp pp	E	E
AB prp	aaa aaa	N	aaa aaa	N	aaa aaa	N	N
	aaa aap	N	aaa aap	N	aaa aap	N	N
	aaa apa	N	aaa apa	N	aaa apa	O	O
	aaa app	N	aaa app	N	aaa app	N	N
	aaa paa	N	aaa paa	N	aaa paa	N	N
	aaa pap	N	aaa pap	N	aaa pap	E	N
	aaa ppa	N	aaa ppa	N	aaa ppa a	E	N
					aaa ppa p	E	**D**
	aaa ppp	F	aaa ppp	N	aaa ppp	E	N
	aap aaa	N	aap aa	M?	aap aaa	N	N
	aap aap	N			aap aap	N	N
	aap apa	N	aap ap	M?	aap apa	N	N
	aap app	N			aap app	N	N
	aap pa	M?	aap paa	N	aap paa	N	N
			aap pap	N	aap pap	N	N
	aap pp	M?	aap pp	M?	aap ppa	N	N
					aap ppp	N	M
	apa aaa	N	apa aaa	N	apa aaa	E	N
	apa aap	N	apa aap	N	apa aap	E	N
	apa apa	N	apa apa	N	apa apa	E	N
	apa app	N	apa app	N	apa app	N	N
	apa paa	O	apa paa	F	apa paa	O	O
	apa pap	N	apa pap	N	apa pap	E	N
	apa ppa	N	apa ppa	N	apa ppa	N	N
	apa ppp	N	apa ppp	N	apa ppp	N	N
	app aaa	N	app aaa	N	app aaa	N	N
	app aap	N	app aap	N	app aap	N	N
	app apa	N	app apa	N	app apa	N	N
	app app	N	app app	N	app app	N	N
	app paa	N	app paa	N	app paa	P	N
	app pap	N	app pap	N	app pap	N	N
	app ppa	O	app ppa	O	app ppa	O	O
	app ppp	O	app ppp	O	app ppp	O	O
	paa aaa	N	paa aaa	N	paa aaa	N	N
	paa aap	N	paa aap	N	paa aap	N	M

	paa ap	**D**	paa ap	**D**	paa ap	**D**	**D**
	paa paa	**D**	paa paa	**F**	paa paa	**D**	**D**
	paa pap	N	paa pap	N	paa pap	N	N
	paa ppa	N	paa ppa	N	paa ppa	N	N
	paa ppp	N	paa ppp	N	paa ppp	N	N
	pap aaa	N	pap aaa	N	pap aaa	N	M
	pap aap	N	pap aap	N	pap aap	N	N
	pap apa	N	pap apa	N	pap apa	N	N
	pap app	N	pap app	N	pap app	N	N
	pap paa	N	pap paa	N	pap paa	N	N
	pap pap	N	pap pap	N	pap pap	N	N
	pap ppa	N	pap ppa	N	pap ppa	N	N
	pap ppp	N	pap ppp	N	pap ppp	N	N
	ppa aaa	O	ppa aaa	O	ppa aaa	O	O
	ppa aap	N	ppa aap	N	ppa aap	N	N
	ppa apa	N	ppa apa	N	ppa apa	N	M
	ppa app	N	ppa app	F	ppa app	N	N
	ppa paa	N	ppa paa	N	ppa paa	N	N
	ppa pap	E	ppa pap	E	ppa pap	E	E
	ppa ppa	F	ppa pp	**D**	ppa pp	**D**	**D**
	ppa ppp	F					
	ppp aaa	N	ppp aaa	N	ppp aaa	N	N
	ppp aap	O	ppp aap	O	ppp aap	O	O
	ppp apa	O	ppp apa	O	ppp apa	O	O
	ppp app	N	ppp app	N	ppp app	N	N
	ppp paa	U	ppp paa	U	ppp paa	U	U
	ppp pap	N	ppp pap	O	ppp pap	O	O
	ppp ppa	O	ppp ppa	E	ppp ppa	O	**D**
	ppp ppp	E	ppp ppp	E	ppp ppp	O	E
MSa	aaa aa	M?	aaa aal	N	aaa aal	N	N
			aaa aar	P	aaa aar	N	N
	aaa apaa	P	aaa apa	F	aaa apaa	P	P
	aaa apap	P			aaa apap		P
	aaa app	P	aaa app	P	aaa app	P	P
	aaa paa	P	aaa paa	P	aaa paa	P	P
	aaa pap	P	aaa pap	P	aaa pap	P	P
	aaa ppa	F	aaa ppa	P	aaa ppa	**D**	**D**
	aaa ppp	P	aaa ppp	P	aaa ppp	P	P
	aap aaa	P	aap aaa	P	aap aaa	P	P
	aap aap	P	aap aap	P	aap aap	P	P
	aap apa	P	aap apa	P	aap apa	P	P
	aap app	P	aap app	P	aap app	P	P
	aap pa	**D**	aap pa	F	aap pa	**D**	**D**
	aap pp	O	aap pp	F	aap pp	O	O
	apa aaa	P	apa aaa	P	apa aaa	P	P
	apa aap	P	apa aap	P	apa aap	P	P
	apa apa	P	apa apa	P	apa apa	P	M
	apa app	P	apa app	P	apa app	P	P
	apa paa	P	apa pa	**D**	apa paa	P	P
	apa pap	**D**			apa pap	**D**	**D**
	apa ppa	P	apa ppa	P	apa ppa	P	P
	apa ppp	O	apa ppp	F	apa ppp	O	O
	app aa	O	app aa	O	app aa	O	O
	app ap	O	app ap	O	app ap	O	O
	app paa	U	app paa	U	app paa	U	U

	app pap	U	app pap	U	app pap	U	U
	app ppa	U	app pp	U	app ppa	U	U
	app ppp	U			app ppp	U	U
	paa aa	M?	paa aa	U	paa aad	N	N
					paa aav	N	N
	paa ap	U	paa ap	U	paa ap	U	U
	paa pa	**D**	paa pa	**D**	paa pa	P	**D**
	paa pp	O	paa pp	U	paa pp	O	O
	pap aa	P	pap aa	U	pap aa	P	M
	pap ap	U	pap ap	U	pap ap	U	U
	pap pa	U	pap pa	U	pap pa	U	U
	pap pp	U	pap pp	U	pap pp	U	U
	ppa aa	F	ppa aa	U	ppa aa	O	O
	ppa ap	O	ppa ap	O	ppa ap	O	O
	ppa pa	**D**	ppa pa	F	ppa pa	**D**	**D**
	ppa pp	U	ppa pp	U	ppa pp	U	U
	ppp aa	U	ppp aa	U	ppp aa	U	U
	ppp ap	U	ppp ap	U	ppp ap	U	U
	ppp pa	U	ppp pa	U	ppp pa	U	U
	ppp pp	U	ppp pp	U	ppp pp	U	U
MSp	aaa aaa	P	aaa aaa	P	aaa aaa	P	P
	aaa aap	**D**	aaa aap	**D**	aaa aap	**D**	**D**
	aaa apa	P	aaa apa	P	aaa apa	P	P
	aaa app	P	aaa app	P	aaa app	P	P
	aaa paa	P	aaa paa	P	aaa paa	E	P
	aaa pap	P	aaa pap	P	aaa pap	E	P
	aaa ppa	P	aaa ppa	P	aaa ppa	P	P
	aaa ppp	P	aaa ppp	P	aaa ppp	E	P
	aap aaa	P	aap aaa	P	aap aaa	P	P
	aap aap	P	aap aap	P	aap aap	**D**	**D**
	aap apa	P	aap apa	P	aap apa	P	P
	aap app	O	aap app	O	aap app	O	O
	aap p	**D**	aap p	**D**	aap p	**D**	**D**
	apa aaa	P	apa aaa	P	apa aaa	P	P
	apa aap	P	apa aap	P	apa aap	P	P
	apa apa	P	apa apaa	P	apa apaa	P	P
			apa apap	P	apa apap	P	**D**
	apa app	P	apa app	P	apa app	P	P
	apa pa	M?	apa paa	P	apa paa	P	P
			apa pap	F	apa pap	P	**D**
	apa ppa	P	apa ppa	F	apa ppa	P	P
	apa ppp	O	apa ppp	O	apa ppp	N	O
	app aa	U	app aa	U	app aa	U	U
	app ap	U	app ap	U	app ap	U	U
	app paa	U	app paa	U	app paa	U	U
	app pap	U	app pap	U	app pap	U	U
	app ppa	U	app pp	U	app ppa	U	U
	app ppp	U			app ppp	U	U
	paa aad	N	paa aad	F	paa aad	N	N
	paa aav	N	paa aav	F	paa aav	N	N
	paa ap	U	paa ap	U	paa ap	U	U
	paa pa	**D**	paa pa	F	paa pa	**D**	**D**
	paa pp	O	paa pp	O	paa pp	O	O
	pap aa	F	pap aa	U	pap aa	P	M
	pap ap	U	pap ap	U	pap ap	U	U

	pap paa	U	pap pa	U	pap pa	U	U
	pap pap	U					
	pap pp	U	pap pp	U	pap pp	U	U
	ppa aa	D	ppa aa	U	ppa aa	P	D
	ppa ap	O	ppa ap	O	ppa ap	O	O
	ppa pa	D	ppa pa	U	ppa pa	D	D
	ppa pp	U	ppa pp	U	ppa pp	U	U
	ppp aa	U	ppp aa	U	ppp aa	U	U
	ppp ap	U	ppp ap	U	ppp ap	U	U
	ppp pa	U	ppp pa	U	ppp pa	U	U
	ppp pp	U	ppp pp	U	ppp pp	U	U
	Ealaaa	I	Ealaaa	I	Ealaaa	I	I
	Ealaap	I	Ealaap	I	Ealaap	I	I
	Ealap	I	Ealap	I	Ealap	I	I
	Ealpa	I	Ealp	D	Ealpa	I	I
	Ealpp	I			Ealpp	I	I
	Earaaa	I	Earaaa	I	Earaaa	I	I
	Earaap	I	Earaap	I	Earaap	I	I
	Earap	I	Earap	I	Earap	I	I
	Earpa	I	Earp	D	Earpa	I	I
	Earpp	I			Earpp	I	I
	Eplaa	I	Eplaa	I	Eplaa	I	I
	Eplap	I	Eplap	I	Eplap	I	I
	Eplpaa	I	Eplpaa	I	Eplpa	I	I
	Eplpap	I	Eplpap	I			
	Eplpp	I	Eplppa	I	Eplppa	I	I
			Eplppp	I	Eplppp	I	I
	Epraa	I	Epraa	I	Epraa	I	I
	Eprap	I	Eprap	I	Eprap	I	I
	Eprpaa	I	Eprpaa	I	Eprpa	I	I
	Eprpap	I	Eprpap	I			
	Eprpp	I	Eprppa	I	Eprppa	I	I
			Eprppp	I	Eprppp	I	I
	Caaaaa	E	Caaaaa	E	Caaaaa	E	E
	Caaaap	E	Caaaap	E	Caaaap	E	E
	Caaapa	E	Caaapa	E	Caaapa	E	E
	Caaapp	E	Caaapp	E	Caaapp	E	E
	Caapaa	N	Caapaa	E	Caapaa	N	N
	Caapap	E	Caapap	E	Caapap	E	D
	Caappa	E	Caappa	E	Caappa	E	E
	Caappp	N	Caappp	E	Caappp	N	N
	Capaaaa	U	Capaaaa	U	Capaaaa	U	U
	Capaaap	U	Capaaap	U	Capaaap	U	U
	Capaapa	U	Capaapa	U	Capaapa	U	U
	Capaapp	U	Capaapp	U	Capaapp	U	U
	Capapaa	U	Capapaa	U	Capapaa	U	U
	Capapap	U	Capapap	U	Capapap	U	U
	Capappa	U	Capappa	U	Capappa	U	U
	Capappp	U	Capappp	U	Capappp	U	U
	Cappaaa	U	Cappaaa	U	Cappaaa	U	U
	Cappaap	U	Cappaap	U	Cappaap	U	U
	Cappapa	U	Cappapa	U	Cappapa	U	U
	Cappapp	U	Cappapp	U	Cappapp	U	U
	Capppaa	U	Capppaa	U	Capppaa	U	U
	Capppap	U	Capppap	U	Capppap	U	U

	Cappppa	U	Cappppa	U	Cappppa	U	U
	Capppppp	U	Capppppp	U	Capppppp	U	U
	Cpaaaa	E	Cpaaaa	E	Cpaaaa	E	E
	Cpaaap	E	Cpaaap	E	Cpaaap	E	E
	Cpaapa	E	Cpaapa	E	Cpaapa	E	E
	Cpaapp	E	Cpaapp	E	Cpaapp	E	E
	Cpapaa	E	Cpapaa	E	Cpapaa	E	E
	Cpapap	E	Cpapap	E	Cpapap	E	E
	Cpappa	E	Cpappa	E	Cpappa	E	E
	Cpappp	E	Cpappp	E	Cpappp	E	E
	Cppaaaa	U	Cppaaaa	U	Cppaaaa	U	U
	Cppaaap	U	Cppaaap	U	Cppaaap	U	U
	Cppaapa	U	Cppaapa	U	Cppaapa	U	U
	Cppaapp	U	Cppaapp	U	Cppaapp	U	U
	Cppapaa	U	Cppapaa	U	Cppapaa	U	U
	Cppapap	U	Cppapap	U	Cppapap	U	U
	Cppappa	U	Cppappa	U	Cppappa	U	U
	Cppappp	U	Cppappp	U	Cppappp	U	U
	Cpppaaa	U	Cpppaaa	U	Cpppaaa	U	U
	Cpppaap	U	Cpppaap	U	Cpppaap	U	U
	Cpppapa	U	Cpppapa	U	Cpppapa	U	U
	Cpppapp	U	Cpppapp	U	Cpppapp	U	U
	Cppppaa	U	Cppppaa	U	Cppppaa	U	U
	Cppppap	U	Cppppap	U	Cppppap	U	U
	Cpppppd	U	Cpppppd	U	Cpppppd	U	U
	Cpppppv	U	Cpppppv	U	Cpppppv	U	U
	Daaaa	U	Daaaa	U	Daaaa	U	U
	Daaap	U	Daaap	U	Daaap	U	U
	Daapa	U	Daapa	U	Daapa	U	U
	Daapp	U	Daapp	U	Daapp	U	U
	Dapaa	U	Dapaa	U	Dapaa	U	U
	Dapap	U	Dapap	U	Dapap	U	U
	Dappaa	U	Dappaa	U	Dappaa	U	U
	Dappap	U	Dappap	U	Dappap	U	U
	Dapppa	U	Dapppa	U	Dapppa	U	U
	Dapppp	U	Dapppp	U	Dapppp	U	U
	Dpaaa	U	Dpaaa	U	Dpaaa	U	U
	Dpaap	U	Dpaap	U	Dpaap	U	U
	Dpapa	U	Dpapa	U	Dpapa	U	U
	Dpapp	U	Dpapp	U	Dpapp	U	U
	Dppaa	U	Dppaa	U	Dppaa	U	U
	Dppap	U	Dppap	U	Dppap	U	U
	Dpppaa	U	Dpppaa	U	Dpppaa	U	U
	Dpppap	U	Dpppap	U	Dpppap	U	U
	Dppppa	U	Dppppa	U	Dppppa	U	U
	Dpppppp	U	Dpppppp	U	Dpppppp	U	U
	P4aa	G	P4a	G	P4a	G	G
	P4ap	G					
	P4pa	G	P4p	G	P4p	G	G
	P4pp	G					

Table 4.2: Table of cells per founder cell and per cell fate

Comparison of the number of terminal cells per tissue type (row) and founder cell (column) between *Rhabditophanes* sp. (*Rh*), *H. gingivalis* (*Hg*), C*. elegans* (*Ce*) and *P. marina* (*Pm*).

Table 4.3: List of studied species and their intestinal pattern

Species are grouped per family. The intestinal pattern of each species is marked with a letter corresponding to the species where this pattern was originally observed: *P. redivivus* (P) or *C. elegans* (C).

Family	Species	Pattern	Remarks
Panagrolaimidae	*Panagrobelus stammeri*	C	
	Panagrellus redivivus	P	
	Halicephalobus gingivalis	P	
	Panagrolaimus rigidus	C	
			gastrulation at 4E cell stage
Alloionematidae	*Rhabditophanes sp.*	C	Ep(l/r)a divides instead of Ep(l/r)p
Cephalobidae	*Cephalobus cubaensis*	C	
Rhabditidae	*Pellioditis marina*	C	No rotation of anterior rings
	Caenorhabditis elegans	C	

Table 4.4: Table of cells per AB great-granddaughter and per fate

Comparison between *Rhabditophanes* sp. (Rh), *H. gingivalis* (Hg), *P. marina* (Pm) and *C. elegans* (Ce).

Table 4.5: List of programmed cell deaths

Rhabditophanes sp. (Rh), *H. gingivalis* (Hg), *P. marina* (Pm) and *C. elegans* (Ce). . The fate of each cell is marked with a letter and color code in accordance with the color code of the cell lineage figures. blue: neuron (N); green: pharynx (P); purple: epidermis (E); darkblue: other fate (O); grey: programmed cell death (D); orange: body muscle (U); dark green: intestine (I); gonad (G); unresolved cell (F); unresolved mitosis (M?); mitosis (M).

		Rh		*Hg*		*Pm*	*Ce*
	name	fate	name	fate	name	fate	fate
AB ala	aaa lar	N	aaa lar	F	aaa lar	N	**D**
	aaa lpa	N	aaa lpa	N	aaa lpa	N	**D**
	aaa rla	N	aaa rla	N	aaa rla	N	**D**
	aaa rra	N	aaa rra	N	aaa rra	N	**D**
	aap arl	N	aap arl	F	aap arl	P	**D**
	aap plr	N	aap plr	N	aap plr	**D**	**D**
	apa aal	N	apa aal	**D**	apa aal	**D**	**D**
	apa apa	N	apa apa	F	apa apa	N	**D**
	apa pa	F	apa pa	F	apa pa	**D**	**D**
	apa ppa	N	apa ppa	F	apa ppa	N	**D**
	app aa	**D**	app aa	**D**	app aa	**D**	**D**
	app pap	N	app pap	N	app pap	**D**	**D**
	app pp	M?	app ppa	F	app ppa p	**D**	**D**
	paa aap	N	paa aa	M?	paa aap	N	**D**
	paa pap	N	paa pap	N	paa pap	**D**	**D**
	paa ppa	N	paa ppa	F	paa ppa p	**D**	**D**
	pap aaa	N	pap aa	F	pap aa	**D**	**D**
	pap apa	N	pap apa	N	pap apap	N	**D**
	pap paa	N	pap paa	N	pap paap	**D**	**D**
	pap pap	N	pap pap	F	pap pap	**D**	N
	ppa aa	**D**	ppa aa	F	ppa aa	**D**	**D**
	ppa app	N	ppa ap	M?	ppa app	**D**	**D**
	ppa pap	N	ppa pap	N	ppa pap	**D**	**D**
	ppp aaa	N	ppp aaa	**D**	ppp aaa	P	N
	ppp apa	N	ppp apa	N	ppp apap	**D**	**D**
	ppp pa	M?	ppp paa	N	ppp paap	**D**	**D**
AB alp	aaa app	P	aaa app	P	aaa app	**D**	**D**
	aaa pap	P	aaa pap	P	aaa pap	**D**	**D**
	aaa ppa	P	aaa ppa	P	aaa ppa	**D**	**D**
	apa app	F	apa app	**D**	apa app	**D**	**D**
	apa ppa	P	apa ppa	**D**	apa ppa	**D**	**D**
	app aaa	F	app aaa	**D**	app aaa	**D**	**D**
	app pap	**D**	app pa	F	app pap	**D**	**D**
	paa aa	**D**	paa aa	**D**	paa aa	**D**	**D**
	paa paa	N	paa pa	**D**	paa pa	**D**	**D**
	paa ppa	N	paa ppa	N	paa ppa	P	**D**
	ppa apv	F	ppa apv	F	ppa apv	P	**D**
	ppa ppa	N	ppa ppa	F	ppa ppa	H	**D**
	ppa ppp	N	ppa ppp	F	ppa ppp	**D**	M
	ppp aa	M?	ppp aav	**D**	ppp aav	**D**	**D**
	ppp app	N	ppp app	**D**	ppp app	**D**	**D**
	ppp ppa	N	ppp ppa	N	ppp ppaa	**D**	N
					ppp ppap	P	**D**
AB ara	aaa paa	F	aaa paa	P	aaa paa	P	**D**
	aaa pp	**D**	aaa pp	**D**	aaa pp	**D**	**D**
	apa paa	P	apa paa	P	apa paa d	P	**D**
	app paa	P	app paa	P	app paa d	P	**D**
	paa app	P	paa app	P	paa app	**D**	**D**
	paa pap	F	paa pap	F	paa pap	N	**D**
	pap aaa	**D**	pap aaa	**D**	pap aaa	**D**	**D**
	pap pap	P	pap pap	P	pap pap	**D**	**D**

	ppa aap	N	ppa aap	F	ppa aap	D	D
	ppa app	H	ppa app	D	ppa app	D	D
	ppa pap	N	ppa pap	N	ppa papa	D	D
	ppp ppa	N	ppp ppap	N	ppp ppap	D	D
AB arp	aaa pp	D	aaa pp	D	aaa pp	D	D
	apa apa	F	apa apa	N	apa apa	D	D
AB pla	aaa apa	N	aaa ap	M?	aaa apa	D	D
	paa aa	M?	paa aaa	N	paa aaa	D	D
	pap ppa	D	pap ppa	H	pap ppa	D	D
	ppp ap	M?	ppp apa	F	ppp apa	D	D
AB plp	aaa ppa	N	aaa ppa	N	aaa ppa p	D	D
	app ap	D	app ap	D	app ap	D	D
	paa ap	D	paa ap	F	paa ap	D	D
	paa paa	N	paa paa	F	paa paa	D	D
	ppa ppa	F	ppa pp	D	ppa pp	D	D
	ppp ppa	O	ppp ppa	H	ppp ppa	O	D
AB pra	aaa apv	F	aaa ap	M?	aaa apv	D	D
	aaa ppa	F	aaa ppa	N	aaa ppa	D	D
	aaa ppp	F	aaa ppp	N	aaa ppp p	D	D
	aap aap	F	aap aap	F	aap aap	H	D
	aap app	N	aap app	N	aap app	N	D
	apa ppp	N	apa ppp	N	apa ppp p	N	D
	paa aaa	N	paa aa	H	paa aaa	D	D
	pap pp	M?	pap ppa	N	pap ppa	D	D
	ppp aap	F	ppp aap	N	ppp aap	D	D
	ppp ap	M?	ppp ap	H	ppp apa	D	D
AB prp	aaa ppa	N	aaa ppa	N	aaa ppa p	H	D
	paa ap	D	paa ap	D	paa ap	D	D
	paa paa	D	paa paa	F	paa paa	D	D
	ppa ppa	F	ppa pp	D	ppa pp	D	D
	ppp ppa	O	ppp ppa	H	ppp ppa	O	D
MSa	aaa ppa	F	aaa ppa	P	aaa ppa	D	D
	aap pa	D	aap pa	F	aap pa	D	D
	apa paa	P	apa pa	D	apa paa	P	P
	apa pap	D			apa pap	D	D
	paa pa	D	paa pa	D	paa pa	P	D
	ppa pa	D	ppa pa	F	ppa pa	D	D
MSp	aaa aap	D	aaa aap	D	aaa aap	D	D
	aap aap	P	aap aap	P	aap aap	D	D
	aap p	D	aap p	D	aap p	D	D
	apa apa	P	apa apap	P	apa apap	P	D
	apa pa	M?	apa pap	F	apa pap	P	D
	paa pa	D	paa pa	F	paa pa	D	D
	ppa aa	D	ppa aa	U	ppa aa	P	D
	ppa pa	D	ppa pa	U	ppa pa	D	D
	Ealpa	I	Ealp	D	Ealpa	I	I
	Earpa	I	Earp	D	Earpa	I	I
	Caapap	H	Caapap	H	Caapap	H	D

Table 4.6: Fate transformations of pharynx cells

Pairwise comparison of all pharynx cells in each species with their equivalent cell in each of the other species. The number of pharynx cells are indicated under each species in the first row. The number of the third row indicates how much of the pharynx cells in each species have an equivalent pharynx cell in each of the other species (equal fate) and how much cells are specific for this species (unequal fate). In the rows below is indicated which cell fate those unequal cells execute in the other species. For example, *Rhabditophanes* sp. has 95 pharynx cells, 83 of those have an equivalent pharynx cell in *H. gingivalis* and 12 have another fate in *H. gingivalis*. Those 12 cells have an unresolved fate (4), an unresolved mitosis (1), undergo apoptosis (2), beocme neuron (4) or another cell tyoe (4) and one becomes muscle.

Table 4.7: Fate transformations of epidermal cells

Pairwise comparison of all epidermis cells in each species with their equivalent cell in each of the other species. The number of epidermis cells are indicated under each species in the first row. The number of the third row indicates how much of the epidermis cells in each species have an equivalent epidermis cell in each of the other species (equal fate) and how much cells are specific for this species (unequal fate). In the rows below is indicated which cell fate those unequal cells execute in the other species.

A. PHARYNX	*Rhabditophanes* sp. 95				*H. gingivalis* 91				*P. marina* 112				*C. elegans* 86			
	Hg	*Pm*	*Ce*	total	*Rh*	*Pm*	*Ce*	total	*Rh*	*Hg*	*Ce*	total	*Rh*	*Hg*	*Pm*	total
equal fate	83	67	79		83	64	76		67	64	65		79	76	65	
unequal fate	12	28	16		8	27	15		45	41	47		7	10	21	
unresolved	4	0	0	4	5	0	0	5	11	8	0	17	2	5	0	7
mitosis	1	1	4	6	3	0	2	5	8	5	7	20	3	3	1	7
cell death	2	7	9	18	0	7	11	18	2	3	11	16	0	1	0	1
epidermis	0	6	0	6	0	6	1	7	0	0	1	1	0	0	6	6
neuron	4	14	3	21	0	14	1	15	23	29	28	80	2	1	14	17
other	4	0	0	4	0	0	0	0	1	3	0	4	0	0	0	0
muscle	1	0	0	1	0	0	0	0	0	0	0	0	0	0	0	0

B. EPIDERMIS	*Rhabditophanes* sp. 86				*H. gingivalis* 90				*P. marina* 131				*C. elegans* 79			
	Hg	*Pm*	*Ce*	total	*Rh*	*Pm*	*Ce*	total	*Rh*	*Hg*	*Ce*	total	*Rh*	*Hg*	*Pm*	total
equal fate	79	82	75		79	78	72		82	78	75		75	72	75	
unequal fate	7	4	11		11	12	18		49	53	56		4	7	4	
unresolved	4	0	0	4	0	0	0	0	3	8	0	11	2	3	0	5
mitosis	1	0	5	6	2	0	5	7	10	11	11	32	1	0	0	1
cell death	1	1	2	4	1	3	6	10	0	0	4	4	0	0	0	0
pharynx	0	0	0	0	0	0	0	0	6	6	6	18	0	1	0	1
neuron	1	1	3	5	6	5	6	17	29	25	32	86	1	3	2	6
other	0	2	1	3	2	4	1	7	1	3	3	7	0	0	2	2

Table 4.8: Fate transformations of neuronal cells

Pairwise comparison of all neuronal cells in each species with their equivalent cell in each of the other species. The number of neuronal cells are indicated under each species in the first row. The number of the third row indicates how much of the neuronal cells in each species have an equivalent neuronal cell in each of the other species (equal fate) and how much cells are specific for this species (unequal fate). In the rows below is indicated which cell fate those unequal cells execute in the other species.

Table 4.9: Fate transformations of programmed cell deaths

Pairwise comparison of all programmed cell deaths in each species with their equivalent cell in each of the other species. The number of programmed cell deaths are indicated under each species in the first row. The number of the third row indicates how much of the programmed cell deaths in each species have an equivalent programmed cell death in each of the other species (equal fate) and how much cells are specific for this species (unequal fate). In the rows below is indicated which cell fate those unequal cells execute in the other species.

C. NEURONS	*Rhabditophanes* sp. 218				*H. gingivalis* 200				*P. marina* 193				*C. elegans* 217			
	Hg	*Pm*	*Ce*	total	*Rh*	*Pm*	*Ce*	total	*Rh*	*Hg*	*Ce*	total	*Rh*	*Hg*	*Pm*	total
equal fate	158	145	173		158	132	164		145	132	155		173	164	155	
unequal fate	60	73	45		42	68	36		48	61	38		44	53	62	
unresolved	19	0	0	19	18	0	0	18	8	16	0	24	13	13	0	26
mitosis	24	0	14	38	17	1	14	32	24	24	10	58	25	30	0	55
cell death	5	19	25	49	0	12	16	28	0	0	11	11	0	1	2	3
epidermis	8	29	1	38	4	25	3	32	1	5	2	8	3	6	32	41
pharynx	0	23	2	25	2	29	1	32	14	14	14	42	3	1	28	32
other	4	2	3	9	1	1	2	4	1	2	1	4	0	2	0	2

D. CELL DEATH	*Rhabditophanes* sp. 21				*H. gingivalis* 24				*P. marina* 67				*C. elegans* 92			
	Hg	*Pm*	*Ce*	total	*Rh*	*Pm*	*Ce*	total	*Rh*	*Hg*	*Ce*	total	*Rh*	*Hg*	*Pm*	total
equal fate	10	19	21		10	19	20		19	19	64		21	20	64	
unequal fate	11	2	0		14	5	4		48	48	3		71	72	28	
unresolved	7	0	0	7	4	0	0	4	11	16	0	27	15	23	0	38
mitosis	1	0	0	1	1	0	0	1	7	4	1	12	9	6	0	15
pharynx	0	2	0	2	2	3	1	6	7	7	0	14	10	11	11	32
epidermis	1	0	0	1	1	0	0	1	1	3	0	4	2	6	4	12
neuron	0	0	0	0	4	0	1	5	22	17	2	41	33	24	11	68
other	0	0	0	0	0	0	0	0	0	0	0	0	2	0	2	4
muscle	2	0	0	2	0	0	0	0	0	1	0	1	0	2	0	2
intestine	0	0	0	0	2	2	2	6	0	0	0	0	0	0	0	0

Figure 4.1: Embryonic cell lineage of *P. marina*

Embryonic cell lineage of *P. marina* until body muscle contraction (550 minutes after first division). Vertical axis represents time. Timeline in minutes after first division. Left branch is anterior daughter, right branch posterior daughter. Terminal cells are colored according to their fate assignment. light blue: neuron; green: pharynx; purple: epidermis; dark blue: other cell type; orange: body muscle; brown intestine. programmed cell deaths are indicated by a cross at the end of the line.

Figure 4.2: Lineage and fate similarities between four studied species.

Lineage similarity is expressed as the percentage of resolved terminal cells in one species that have the same lineage history in the other species. Fate similarity is expressed as the percentage of cells of the first species that has the same cell fate as the equivalent cell in the second species. Lineage similarities are marked in red, fate similarities in black. The direction of the arrow indicates how the comparison was performed. The number of resolved terminal cells in the lineage of each species is indicated between brackets.

Figure 4.3: Embryonic cell lineage of *Rhabditophanes* sp.

Embryonic cell lineage of *Rhabditophanes* sp. until body muscle contraction (410 minutes after first division). Vertical axis represents time. Timeline in minutes after first division. The AB cell lineage is cut of after 330 min, after the last division round, since not every cell could be followed until body muscle contraction. Left branch is anterior daughter, right branch posterior daughter. Terminal cells are colored according to their fate assignment. light blue: neuron; green: pharynx; purple: epidermis; dark blue: other cell type; orange: body muscle; brown intestine. programmed cell deaths are indicated by a cross at the end of the line.

Figure 4.4: Embryonic cell lineage of *H. gingivalis*

Embryonic cell lineage of *H. gingivalis* until body muscle contraction (650 minutes after first division). Vertical axis represents time. Timeline in minutes after first division. The AB cell lineage is cut of after 490 min, after the last division round, since not every cell could be followed until body muscle contraction. Left branch is anterior daughter, right branch posterior daughter. Terminal cells are colored according to their fate assignment. light blue: neuron; green: pharynx; purple: epidermis; dark blue: other cell type; orange: body muscle; brown intestine. programmed cell deaths are indicated by a cross at the end of the line.

Figure 4.5: The schematic reconstruction of the cell lineage of E.

(A) *H. gingivalis* and *P. redivivus.* (B) *P. marina, Rhabditophanes* sp., *C. elegans*, *C. cubaensis*, *P. stammeri* and *P. rigidus*. Left branch is anterior (a) or left (l) daughter; right branch is posterior (p) or right (r) daughter. X, programmed cell death. Numbers mark the subsequent intestinal rings in the embryonic intestine. L, left cell; R, right cell of the ring. The asterisks mark the cells Ep(l/r)pa that divide in *Rhabditophanes* sp instead of their posterior daughter cells Ep(l/r)pp.

Figure 4.6: DIC images of intestinal morphogenesis in *H. gingivalis*

DIC photographs of the subsequent stages of the *P. redivivus* pattern of intestinal development in the H. gingivalis embryo. Ventral view, anterior to the left; except F: left lateral view. Cells are outlined according to colors in Fig. 1. Black, MSap descendants; grey: MSpp descendants. Visible nuclei are marked with dashed circles. Time (t) is indicated as minutes after first division; optical section (l) as a number from 01 to 25, with 01 as most ventral level and 25 most dorsal level.(A) 2E stage (t=113 min; level=04); note that Ea (blue) is positioned in between MSap (black) and MSpp (grey). (B) 4E stage (t=160 min; level=07); here Eaa and Eap are positioned in between MSap and MSpp daughters. (C) 8E stage (t= 270 min; level=12). (D) 6E-stage (t=360 min; level=14); Bilateral symmetry is established by migration of Eaaa (blue to the left side of the primordium). (E) 12E stage (t=430min; level=15); second pair Ea(a/p)ap and last pair Ep(l/r)pp lie more ventrally than other cells and are not visible in this focal plane. (F) 18E-stage (t=635 min; level=08), left lateral view of comma stage embryo; nine subsequent rings are numbered.

Figure 4.7: 3D-reconstruction of two intestinal patterns

(A-F) *H. gingivalis* and *P. redivivus;* (G-K) *P. marina, Rhabditophanes* sp., *C. elegans*, *C. cubaensis*, *P. stammeri* and *P. rigidus*. Dorsal lateral view from the left. Spheres represent the individual cells, based on the 3D positions marked during lineage analysis. Colored cells are E descendants, colored according to the cell lineages shown in Fig. 4.5. Black, MSap descendants; grey: MSpp descendants. Lines in 3D-reconstructions connect daughter cells. In F and K, the 9 rings in the intestine are numbered with their corresponding lineage, shown in Fig. 4.5.

Figure 4.8: Mapping of intestinal patterns on molecular phylogeny

The species with known intestinal lineage and 3D arrangement were mapped onto a molecular phylogeny based on aligned 18S rDNA. Summary cladogram obtained from maximum likelihood and Bayesian analysis. Branch support indicated by bootstrap values (calculated by 100 replicates) for ML (above branch), and Bayesian probabilities values (below branch), all expressed as percentage.. Species with the *C. elegans* pattern are indicated with square; species with the *P. redivivus* intestinal pattern indicated with star. Outgroup: *Plectus aquatilis* and *Mononchus truncatus*. Corresponding families are to the right. Roman numbers IV and V indicate clades according to the classification of Blaxter et al. (1998).

Figure 4.9: DIC image of the four primordial germ cells in *Rhabditophanes* sp.

Ventral view of 176 cell stage (176 min), anterior to the left. The four primordial germ cells are marked with an asterisk. Arrowhead indicates the blastocoel, the space left after the inward migration of the intestinal cells. scale bar is 10µm.

Figure 4.10: Somatic gonadal cells attached to germline in *Rhabditophanes sp.*

DIC image from -stage. Vental view. Scalebar is 5µm. The somatic gonadal precursors MS(a/p) ppa ap lie adjacent to the four germline precursors derived from P_4 just before elongation.

Figure 4.11: 3D reconstruction of primordial gonad in *Rhabditophanes* sp.

At muscle contraction (t=410 min). Balls represent the position of the nuclei. brown: E; blue: P4; dark green: Msa ppa ap, light green: MSp ppa ap. Note that the primordal gonad is closely associated with the fifth intestinal ring.

Figure 4.12: Comparison of fate distribution in 8AB daughters.

Cell lineage of AB shown here is the one of *Rhabditophanes* sp. The lineages of the 8 granddaughters are oriented according to their spatial position in the embryo (dorsal view, anterior to the left). Terminal cells and precursor cells that give rise to one tissue type are colored according to cell fate: pharynx, green; neuron, blue; epiderm, red; body muscle, yellow, other, dark blue. Cell deaths or cell with unresolved cell fate are colored black. The fate distribution of each AB granddaughter is compared between *Rhabditophanes* sp. (R), *H. gingivalis* (H), *P. marina* (P) and *C. elegans* (C) in percentage of cells of each cell fate to the total number of terminal cells in each granddaughter.

Figure 4.13: 3D reconstruction of pharynx primordium in *Rhabditophanes* sp.

Figure 4.14: Schematic reconstruction of body muscle

Schematic reconstruction of the four body muscle strips at the onset of body muscle contraction in *P. marina* and *Rhabditophanes* sp. Cylindrical projection, viewed from within the animal. The shapes of the muscles are not intended to be realistic, but each cell is defined uniquely by its position in the pattern. The extra cell division of MSp pap pa that forms the extra body muscle cell in *Rhabditophanes* sp. is marked with an asterisk. Cells are colored by lineage origin. Green: AB; Dark grey: MSa: light grey: MSp; dark blue: Da; light blue: Dp; red: Cap; orange: Cpp.

Movie 4.1: Animation of gastrulation and intestinal morphogenesis.

Animations are based on the 3D-reconstructions of the 24 nuclei of the embryo. Left lateral view. Colored cells are E descendants, colored according to the cell lineages shown in Fig. 1. Black, MSap descendants; grey: MSpp descendants. Other cells are colored in white in the embryo, to emphasize the size of the embryo. Lines connect daughter cells after cell division. At the end of the animation, the 9 rings in the intestine are numbered with their corresponding lineage in Fig 4.5. Time in left upper corner is the time after the first division of the zygote. **A.** *P. redivivus.* Note that Ea and its descendants stay in between MSap (black) and MSpp (grey) during gastrulation. Eaap and Eapp undergo programmed cell death (marked with +) and Eaaa migrates to the left side to restore the bilateral symmetry (marked with arrow). **B.** *P. stammeri*, which has a *C. elegans* intestinal pattern. Here, Ea migrates inwards before MSap and MSpp and divides left-right to establish early bilateral symmetry in the intestine. The daughters of Ealp (light blue) and Earp (light green) are situated more ventrally and migrate between the other cells to form the second and fifth intestinal ring (marked by arrows).

Movie 4.2: Contralateral migration of dorsal epidermal nuclei.

Based on 3D reconstructions of *Rhabditophanes* sp. Dorsal aspect, anterior upwards. Time is indicated as minutes after division of AB. Cells are colored by lineage origin: purple:ABarp; green: ABpla; red; Caa; orange: Cpa. Cells are numbered according to analogues figure in Sulston et al. (1983) for better comparison. 1. ABarp apa pa; 2. ABpla aaa pa; 3. ABarpaap aa; 4. ABpla aaa pp; 5. ABarp apa pp; 6. ABarp aap ap; 7. ABarp aap pa; 8. ABarp aap pp; 9. ABarp ppa pa; 10. ABarp paa pa; 11, 13, 15, 17. Cpaa; 12,14,16,18: Caaa. Sister cells are connected with lines in the first frame.

CHAPTER 5: DISCUSSION

Table 5.1: Division sequence of intestinal and germline

Comparision of the divsion sequence of the intestinal line (EMS,E) and the germline (P_2, P_3) between species studied here and data available in literature (Laugsch and Schierenberg, 2004; Lahl et al., 2003). Species are grouped per clade according to Blaxter et al. (1998). Divisions that vary within a species are grouped in a square. In the last row is indicated which germline cells show a reversal of polarity (they arise from the anterior daughter instead of the posterior). Note that in all species where EMS divides before or during the division of P2, there is a double reversal of polarity, the outgroup *Plectus* sp. being an exeption.

	CLADE V					CLADE IVa		CLADE IVb	OUT GROUP
	C. elegans	*P. marina*	*R. terricola*	*R. dolichura*	*R. belari*	*Rhabditophanes sp*	*H. gingivalis*	*A. nanus*	*Plectus*
	EMS	EMS	EMS	**P_2**	**P_2**	EMS/ **P_2**	**P_2**	**P_2**	**P_2**
	P_2	**P_2**	**P_2**	EMS	EMS		EMS	**P_3**	EMS
	MS	MS	**P_3**	**P_3**	**P_3**	MS/E	MS	EMS	MS
	E	E	MS	MS	MS		**P_3**	MS	**P_3**
	P_3	**P_3**	E	E	E	**P3**	E	E	E
reversal	**P_2/P_3**	**P_2/P_3**	**P_2/P_3**	**P_2**	**P_2**	**P_2/P_3**	**P_3**	no	**P_2/P_3**

Figure 5.1: Polarity reversal in the germline

Drawings of first divisions illustrating the polarity of the germline divisions. Left lateral view, anterior to the left. Black circles indicate the P-granules that segregates in the germline. Big circles in cells indicate the nuclear envelope, small circles indicate the position of the centrosomes and the future division axis of the next division. Arrows indicate the movement of the nucleus to one side of the cell before division, to generate an asymmetric division.

A-E: *C. elegans;* colored edges indicate cortical proteins that generate the anterior-posterior polarity in cells. Red: cortical PAR-3/PAR-6/PKC-3; blue cortical PAR-1/PAR-2; green: cortical MES-1. **A**. 1-cell stage; **B**. 2-cell stage; **C**. 4-cell stage; **D**. 8-cell stage; **E**. 24-cell stage, the AB progeny is indicated by outline.

F-J: *H. gingivalis;* **F**. 1-cell stage; **G**. 2-cell stage; **H**. 4-cell stage; **I**. 7-cell stage; **J**. 14-cell stage, the AB progeny is indicated by outline.

K-O: *A. nanus*. **K**. 1-cell stage; **L**. 2-cell stage; **M**. 3-cell stage; **N**. 4-cell stage; **O**. 6-cell stage. Green numbers in squares indicate the potential fate each cell can execute. 1:AB-fate; 2: EMS-fate; 3: C-fate. Red number: when this fate is inhibited by a signal from the neighbouring cell in the intact embryo (based on drawings of Wiegner and Schierenberg, 1999).

Figure 5.2: Polarity reversal in germline plotted on phylogeny

Phylogeny based on De Ley and Blaxter (2002). Uppercase names represent the name of the orders (-da) and infraorder (-morpha) of each branch. The clades as defined by Blaxter et al. (1998) are indicated as roman number above the branch. Species from which early developmental data is available are named below each (infra)order. The anterior-posterior order of the early blastomeres are indicated next to each name to show the absence or presence of reversal of polarity in the germline divisions. Species with only partial or no reversal of polarity are indicated with an asterisk. Drawings of early embryos illustrate the configuration of the early blastomeres; Rhabditomorpha: *C. elegans*; Panagrolaimomorpha: *H. gingivalis*. Cephalobomorpha: *A. nanus* (based on Wiegner & Schierenberg, 1999). Drawings of other orders from Malakhov (1994). In clade I: the early divisions are variable and have no defined pattern (Voronov and Panchin, 1998; Schierenberg, 2005).

Figure 5.3: Cell cycle patterns in terms of available gene products

(a-d) Observed changes in cell cycle lengths of the AB cells in four nematode species. *R. terricola* behaves similarly to *R. dolichura* except for two extra long cell cycles. **(A-D)** Proposed decrease of maternal and increase of zygotic components (figure from Laugsch and Schierenberg, 2004).

Figure 5.4: Cell cycle length of AB generations

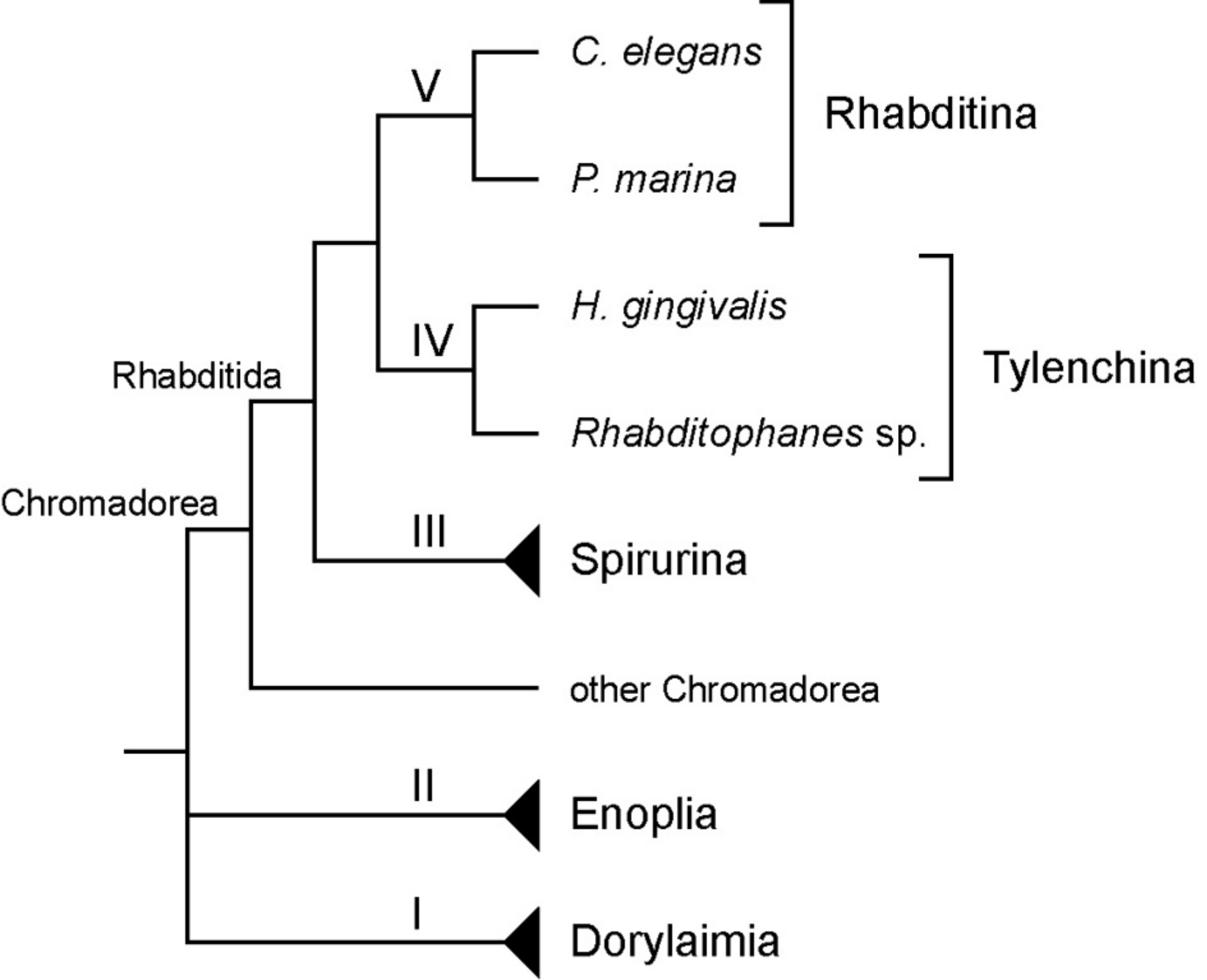
The median length of AB cells in each generation over time of the species studied here is compared with data available in Laugsch and Schierenberg (2004). Note the long cleavage arrest in the 4AB stage of *R. dolichura*.

Figure 5.5: Correlation between cell contacts and symmetry breakage and formation of excretory cell.

DIC images of *Rhabditophanes* sp.scalebar is 10 µm. **A**. 28-cell stage showing cell-cell contact between ABalap and ABplaa. ventral view, anterior to the left. **B**. Same embryo in 375-cell stage illustrating secondary symmetry between ABplaaa (circles) and ABarpap (asterisks). dorsal view, anterior to the left. **C**. 32-cell stage embryo showing cell-cell contact between ABplpa and MSap. ventral view, anterior to the left. **D**. Same embryo in comma stage showing the large excretory cell (arrowhead). Left lateral view, anterior to the left.

Figure 5.6: Monoclonal vs. polyclonal cell lineages

(A-C) Evolutionary transformation of the Caa-lineage. Individual cells in uniform lineage blocks undergo fate transformation or apoptosis. (**A**) *H. gingivalis*; (**B**) *P. marina;* (**C**) *C. elegans*. Embryos in left lateral view at initiation of muscle contraction. (**D-F**) Analysis of migration of the tail neuron precursor in the three species indicates fate transformation allows cells to originate close to the final destination, eliminating unnecessary migrations and allowing faster embryonic development. Dorsal view, anterior to the left. (**D**) *H. gingivalis* sp. 220-455 minutes; (**E**) *P. marina* 200-419 minutes; (**F**) *C. elegans* 159-245 minutes.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Rhabditina
H. gingivalis
IV
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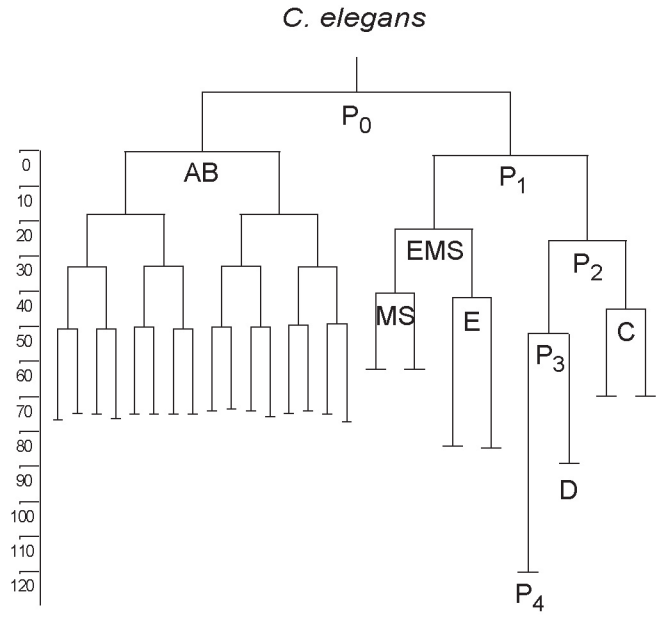
Spirurina
other Chromadorea
II
Enoplia
I
Dorylaimia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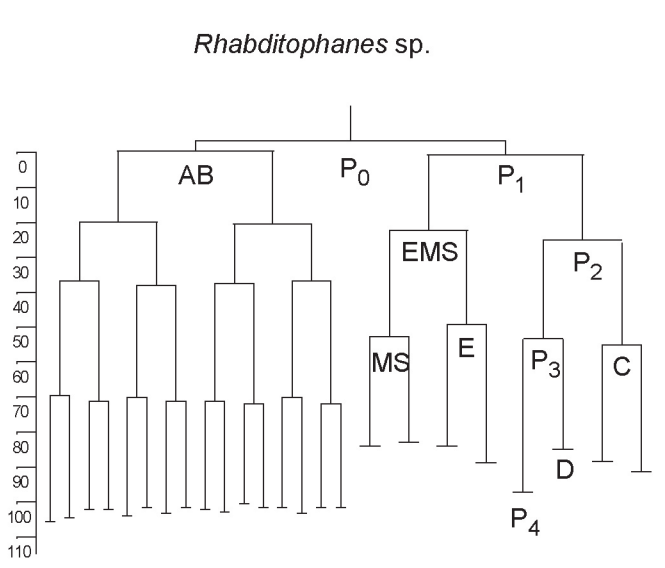
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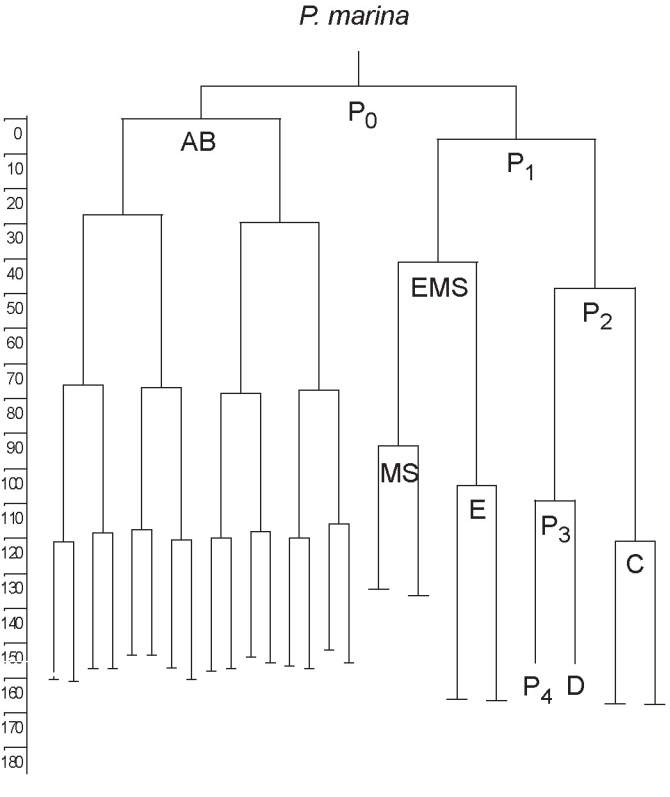
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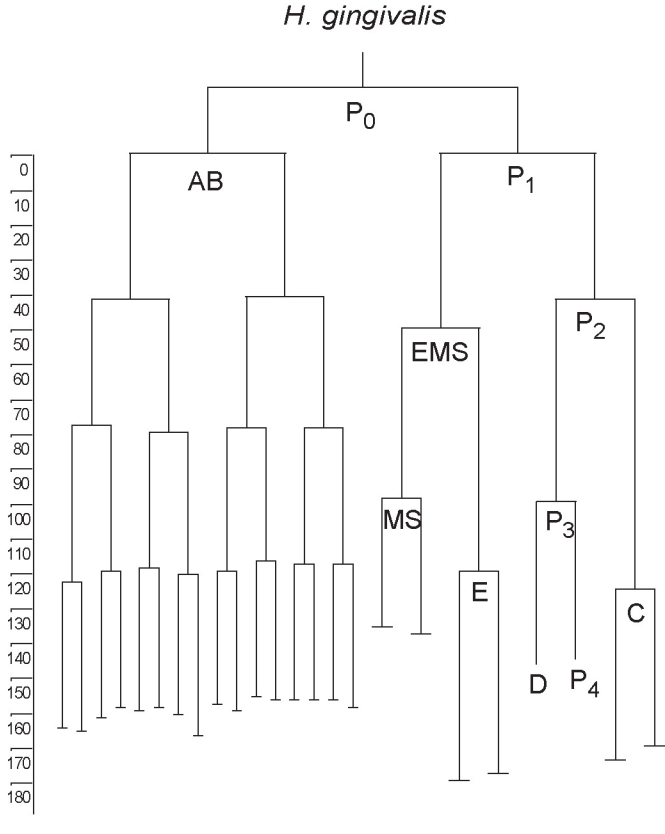
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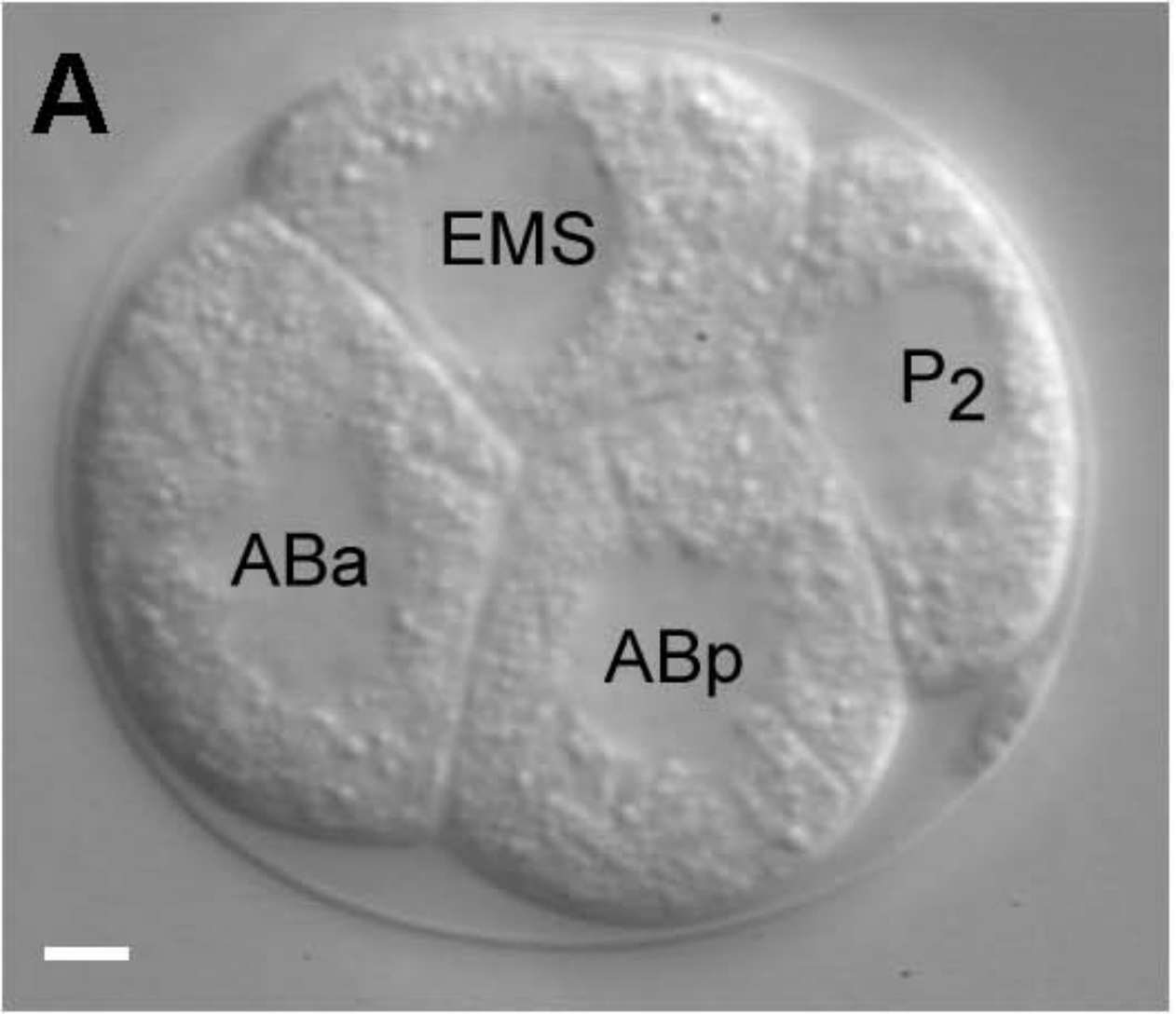
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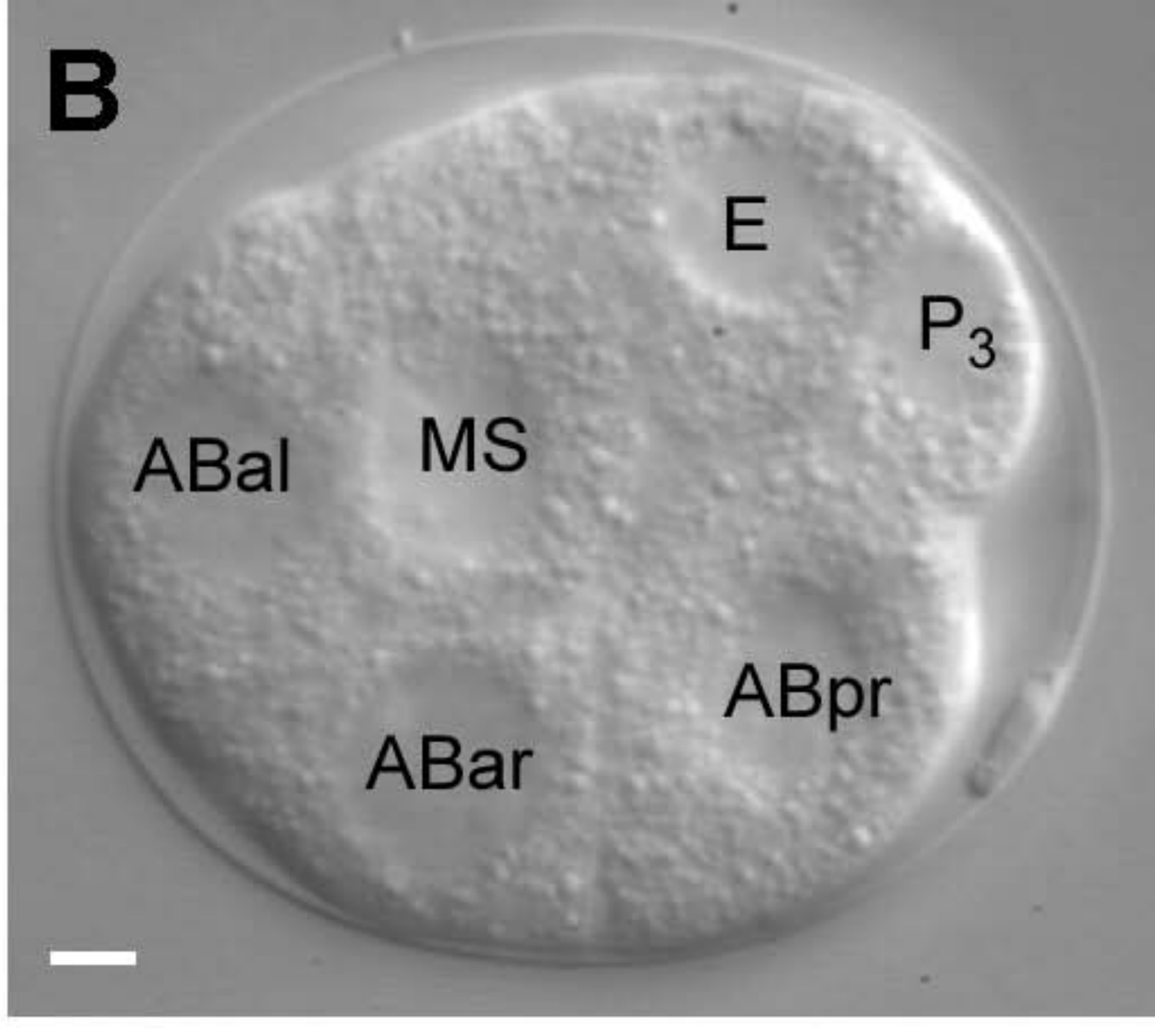
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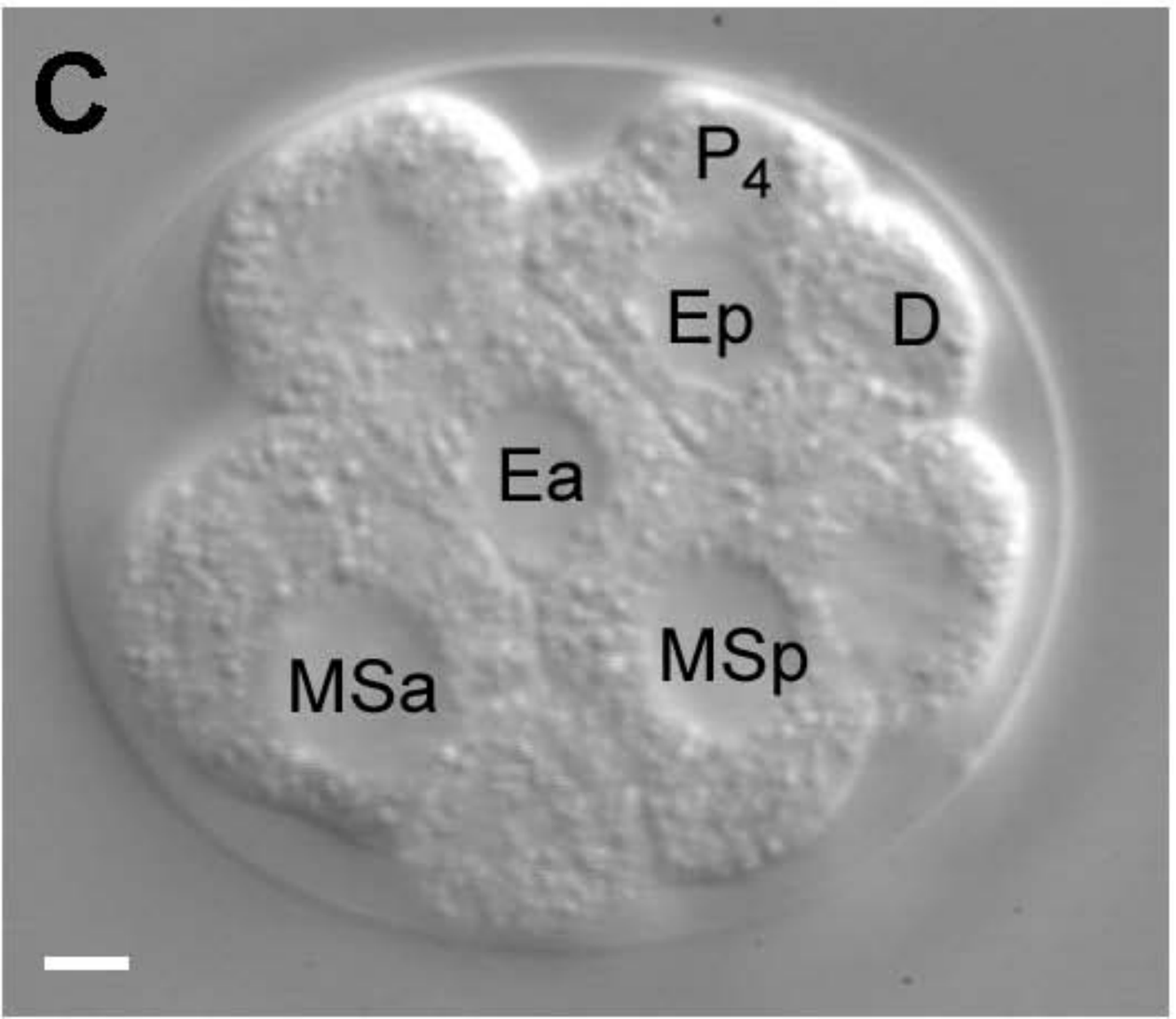
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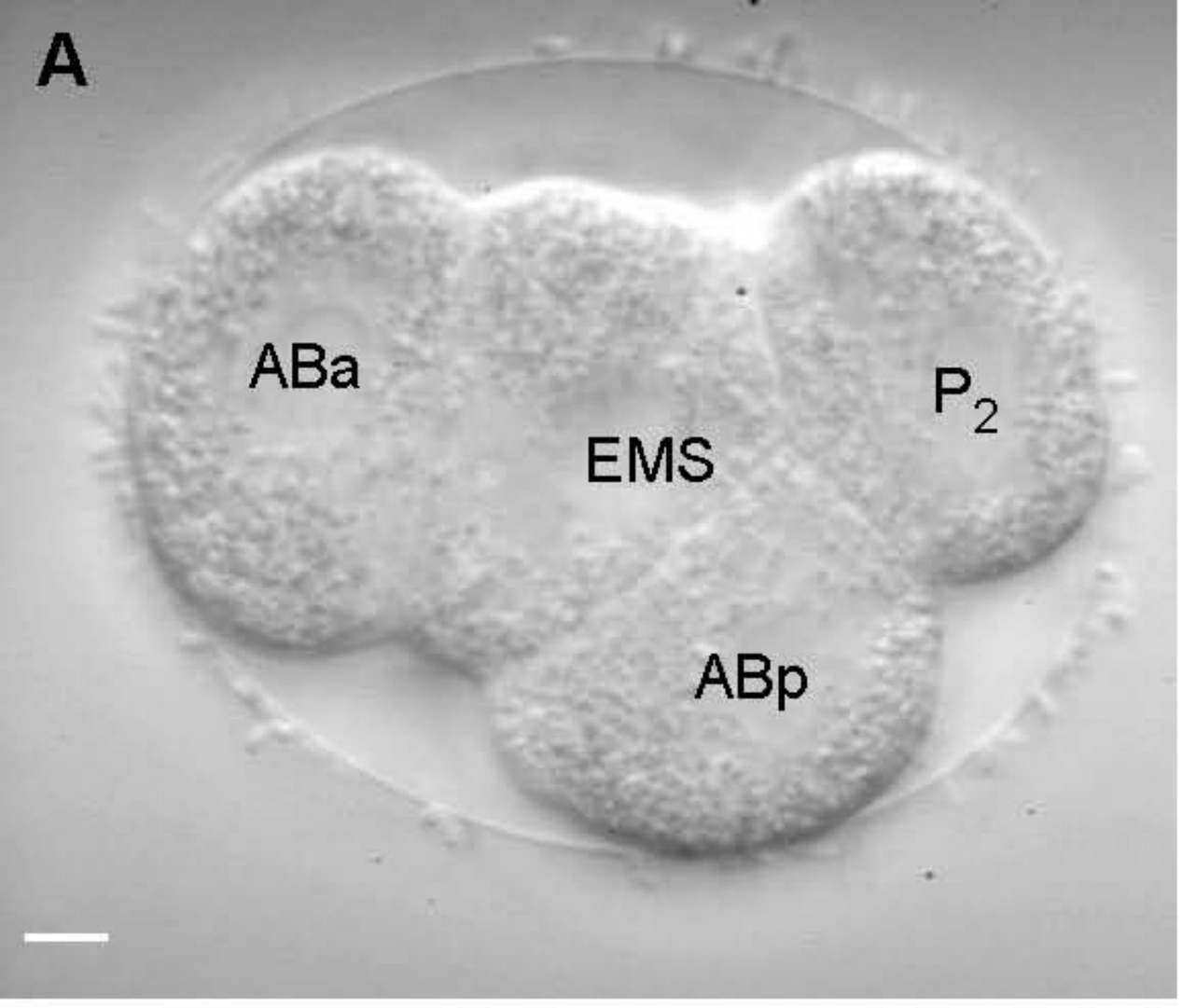
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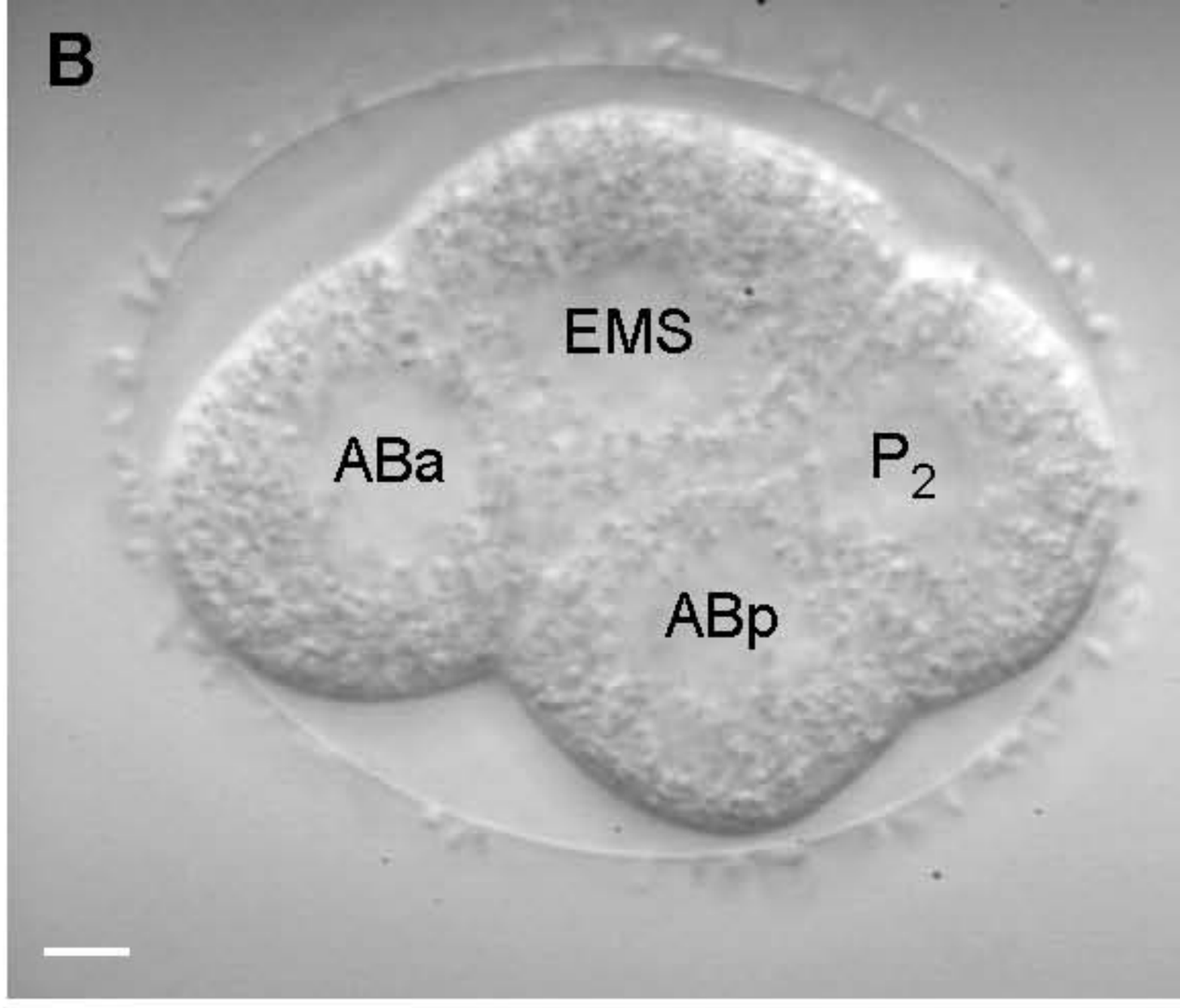
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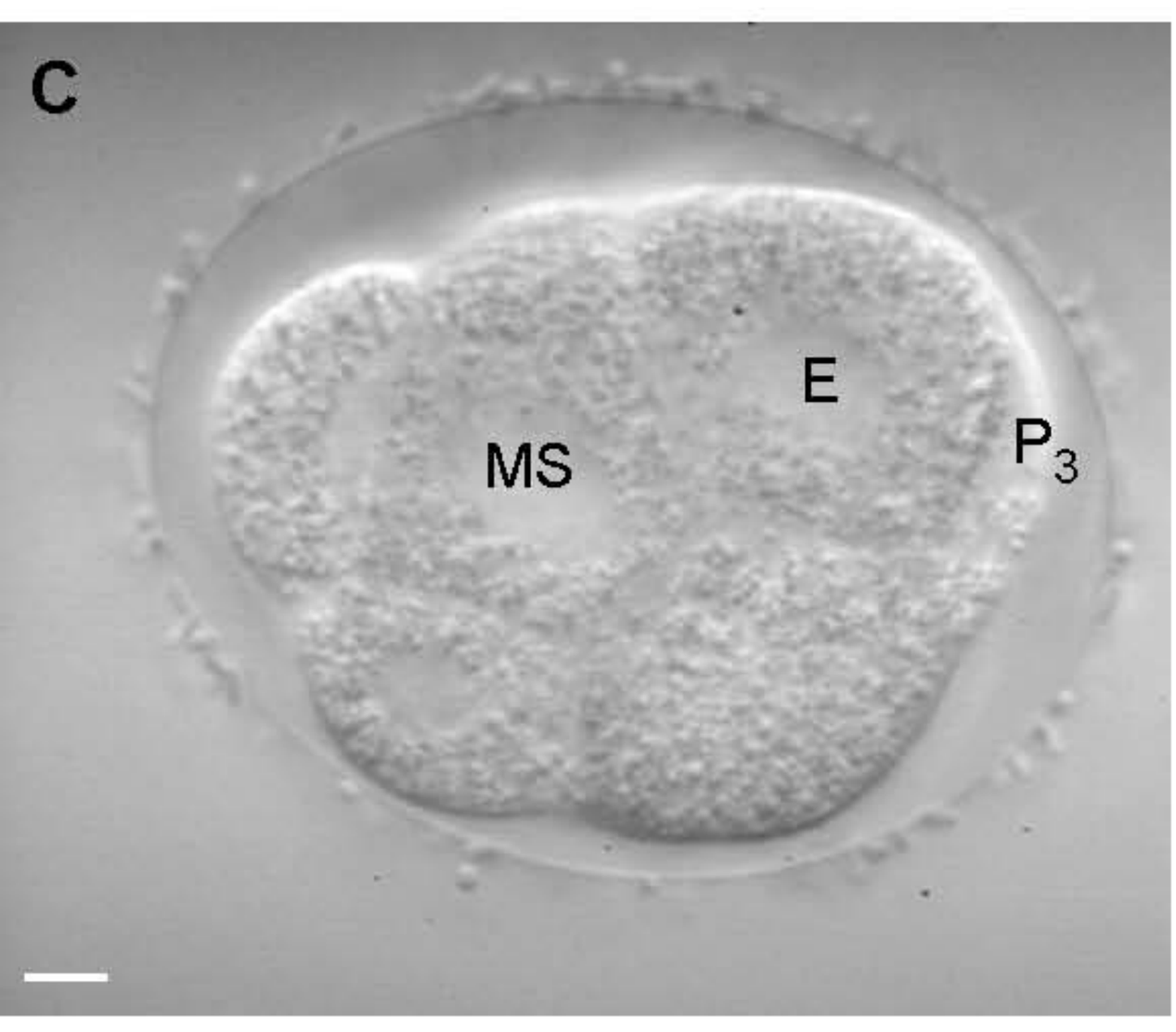
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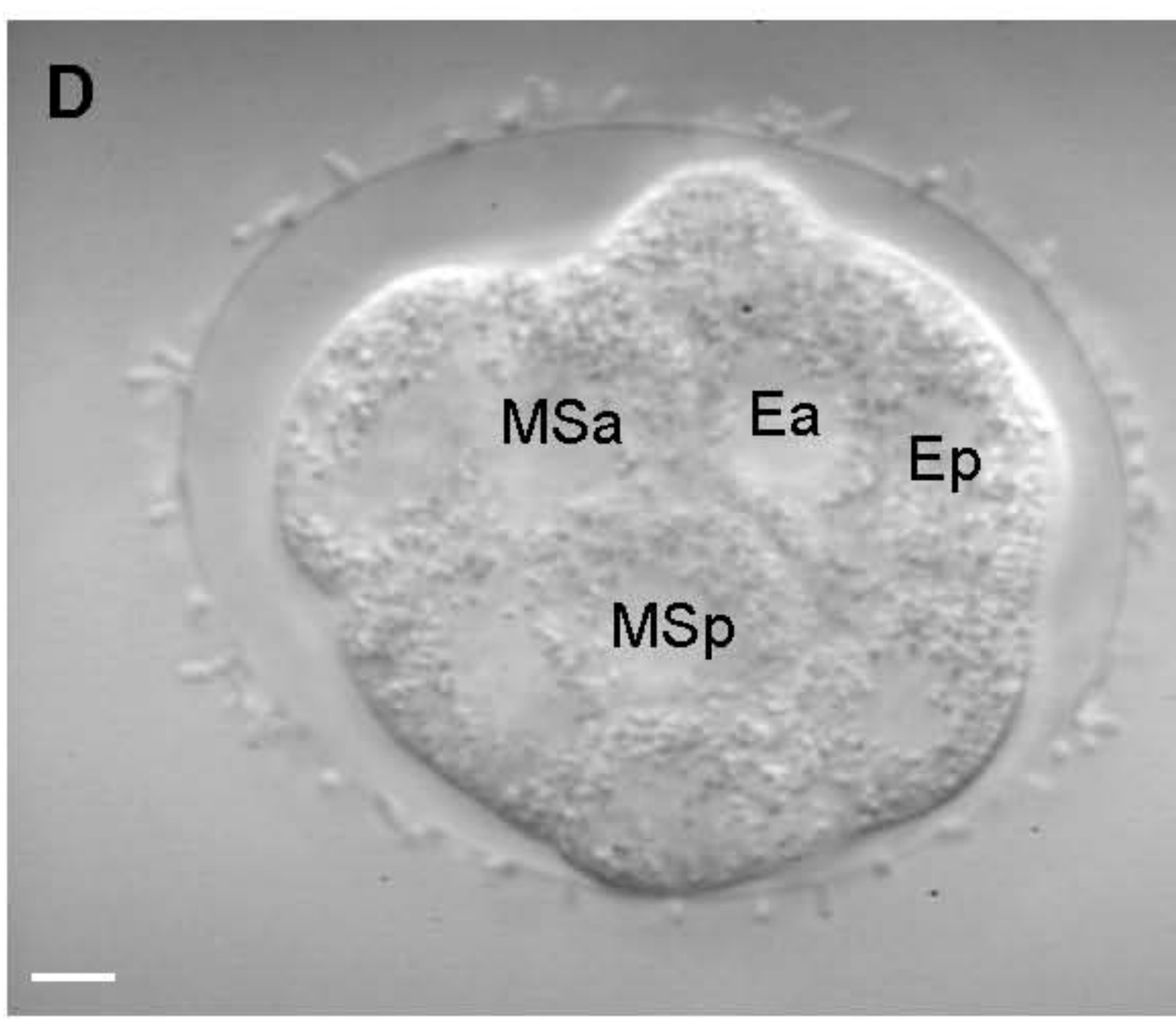
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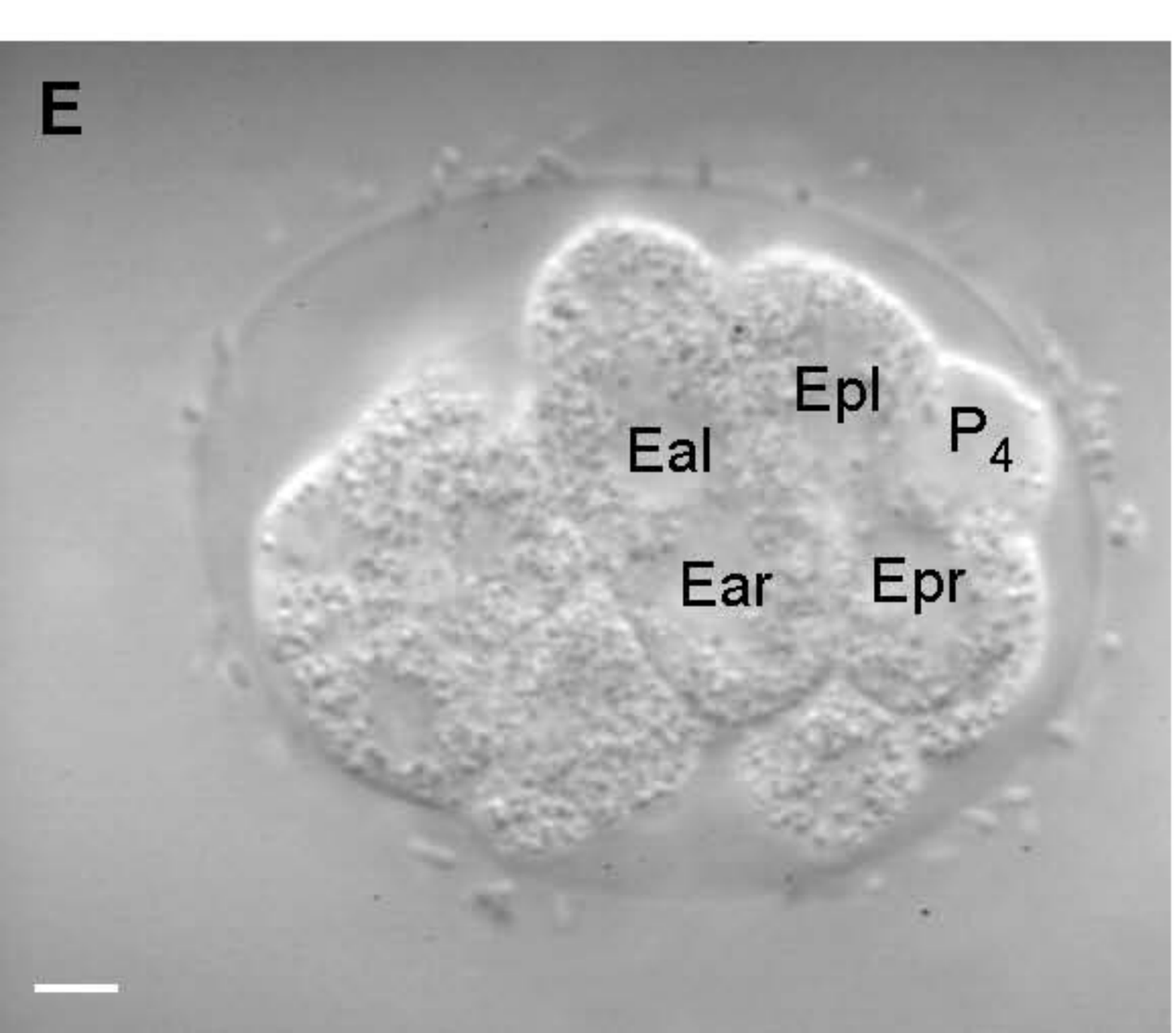
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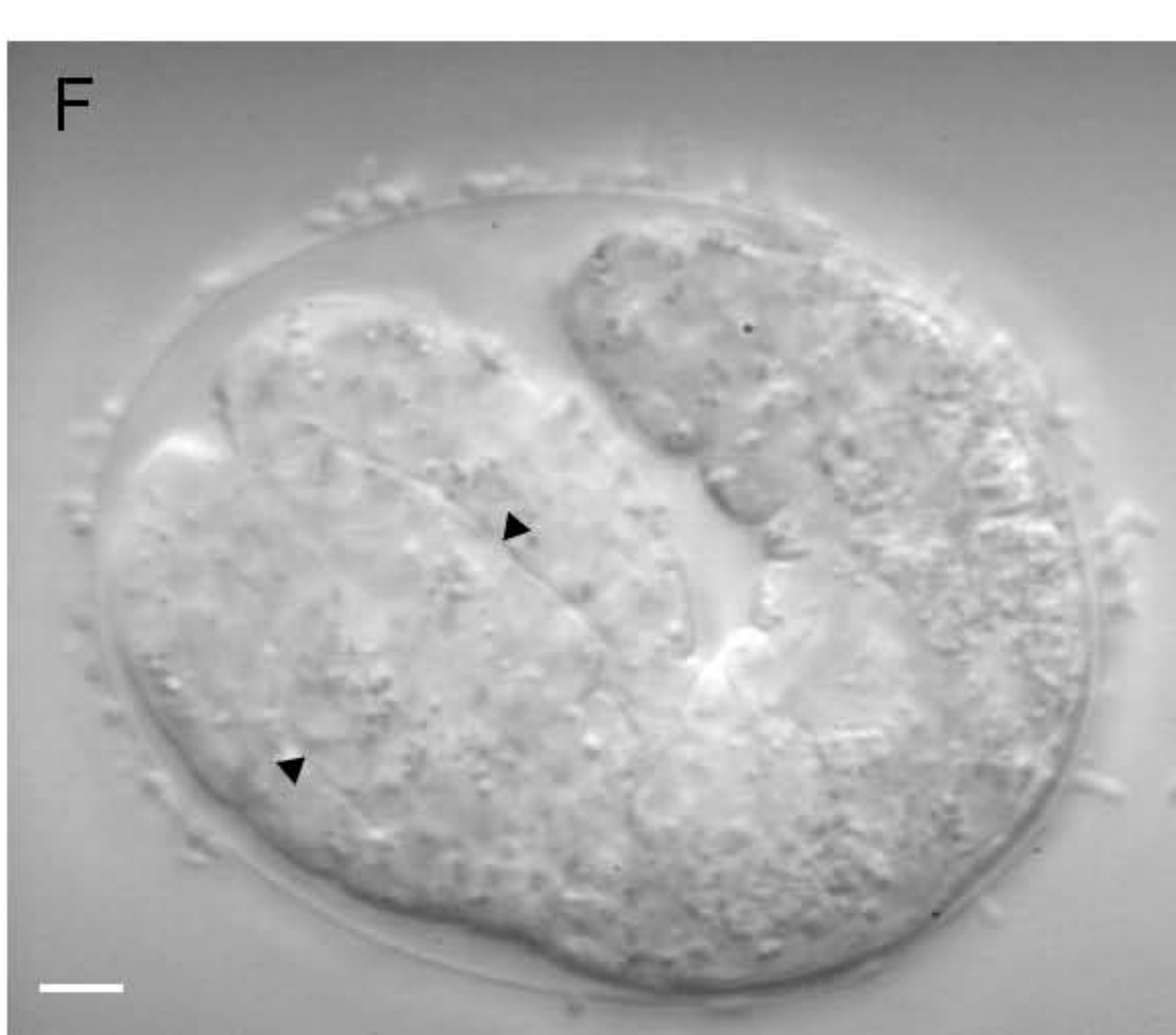
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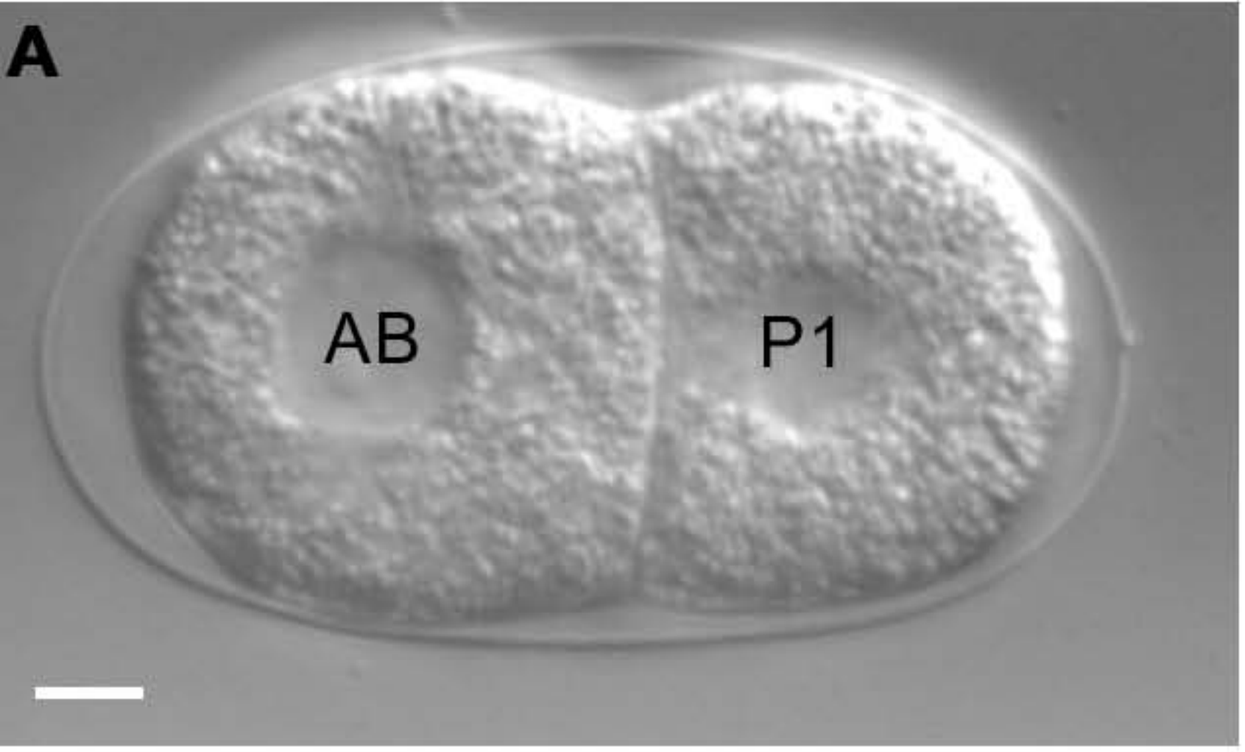
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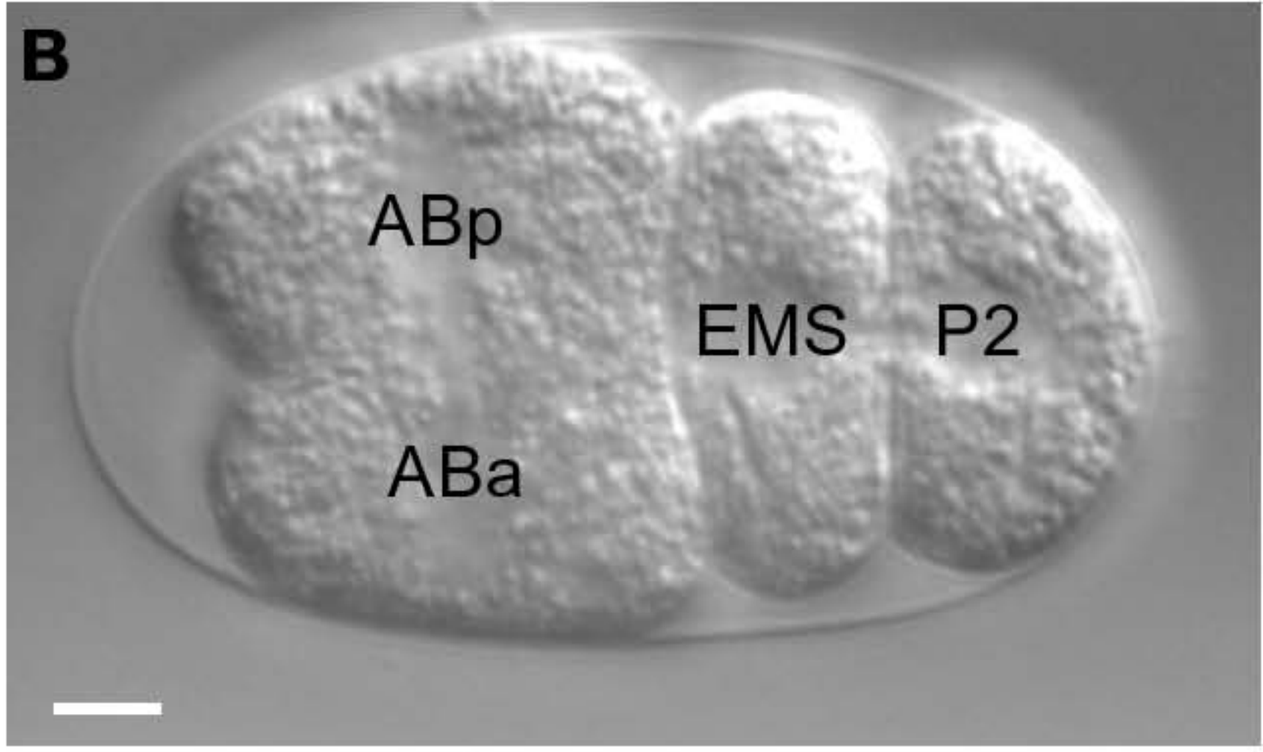
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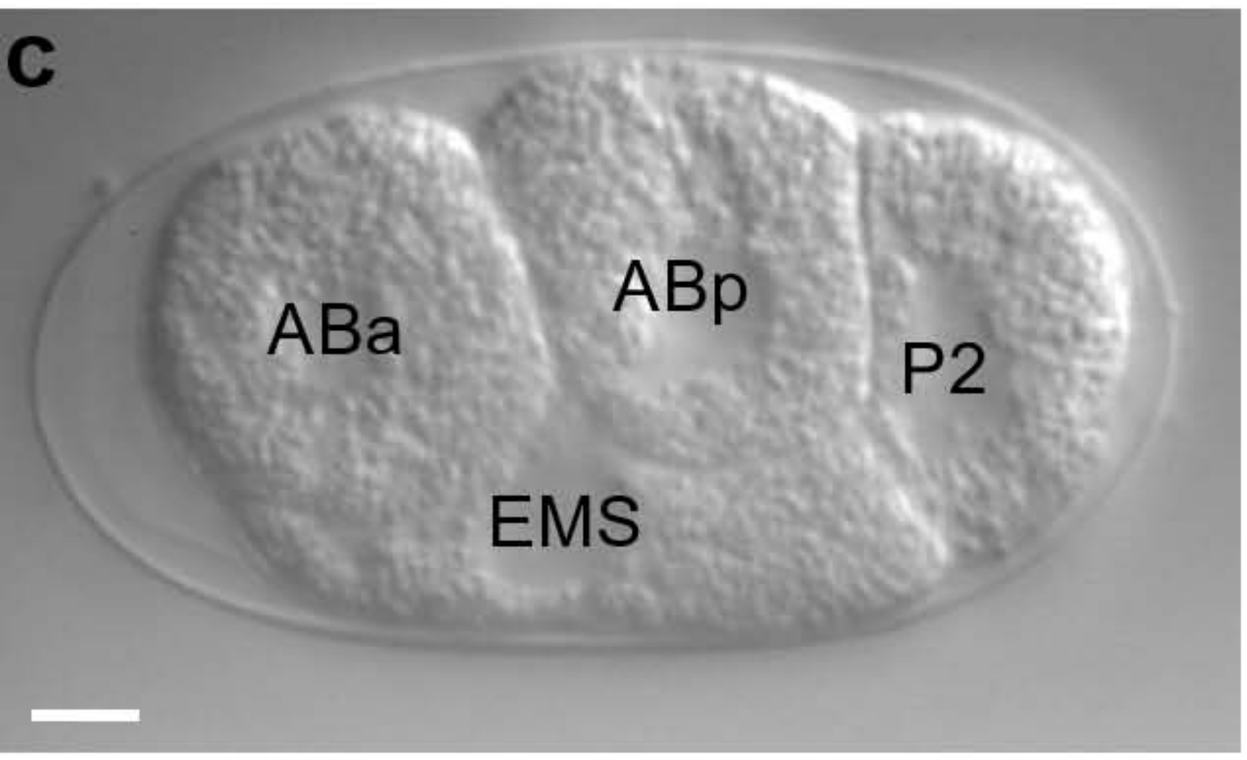
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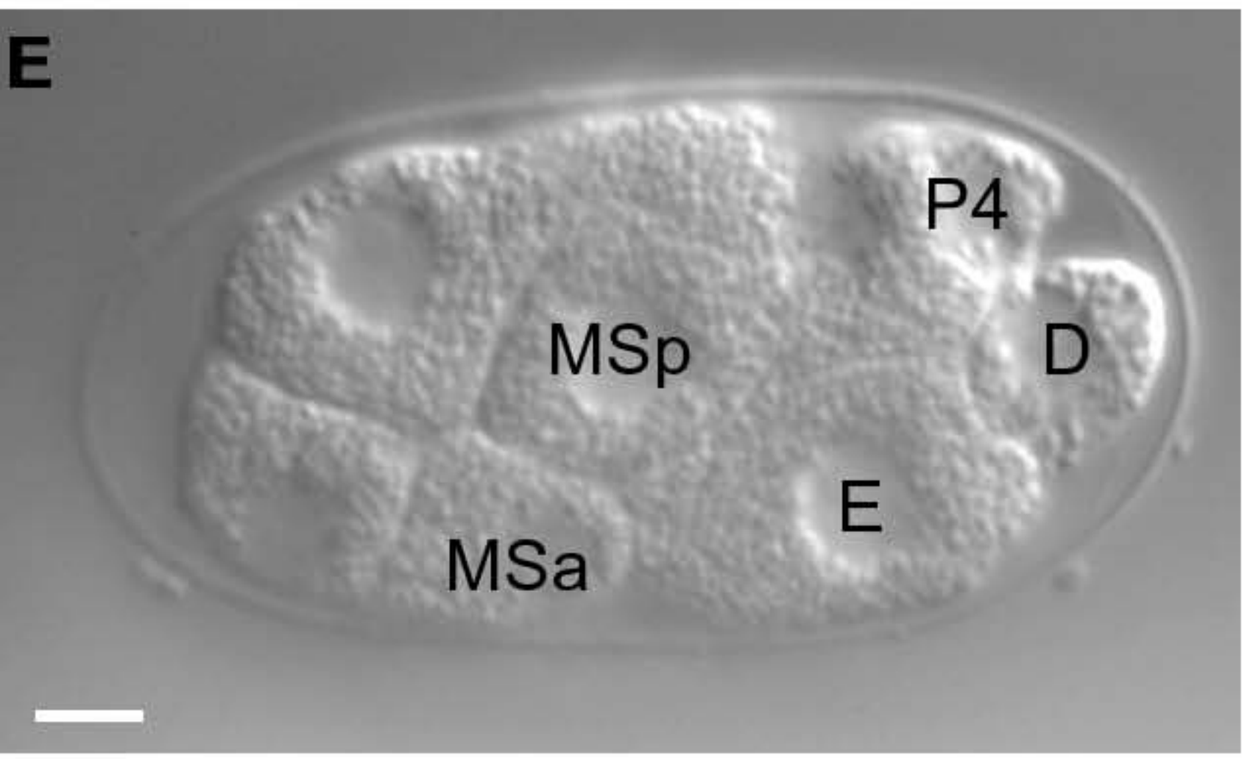
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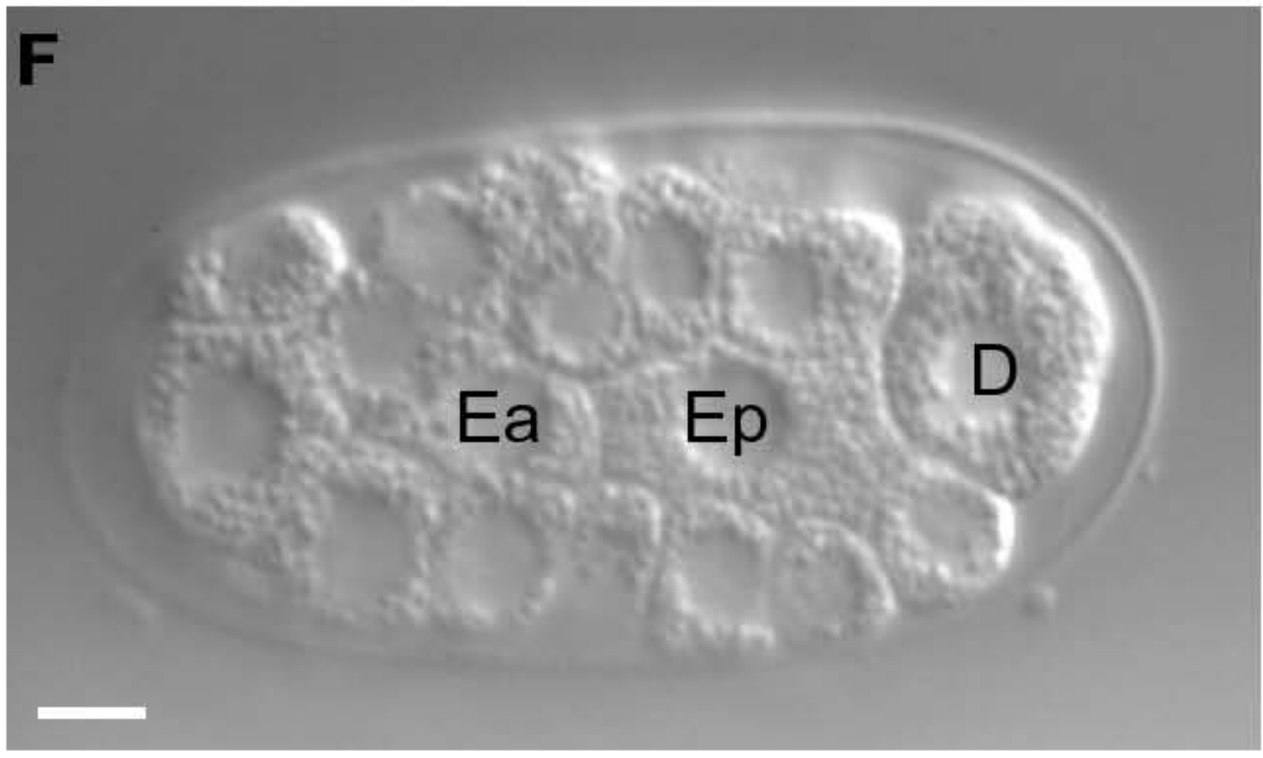
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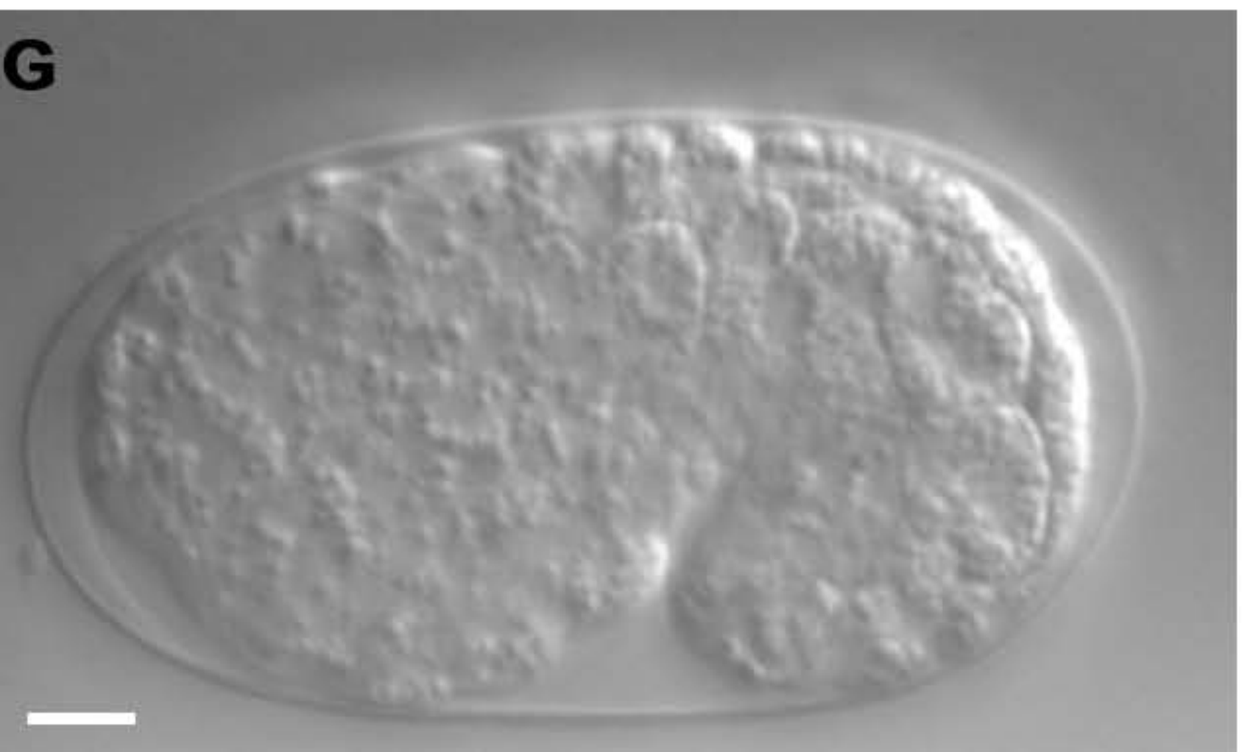
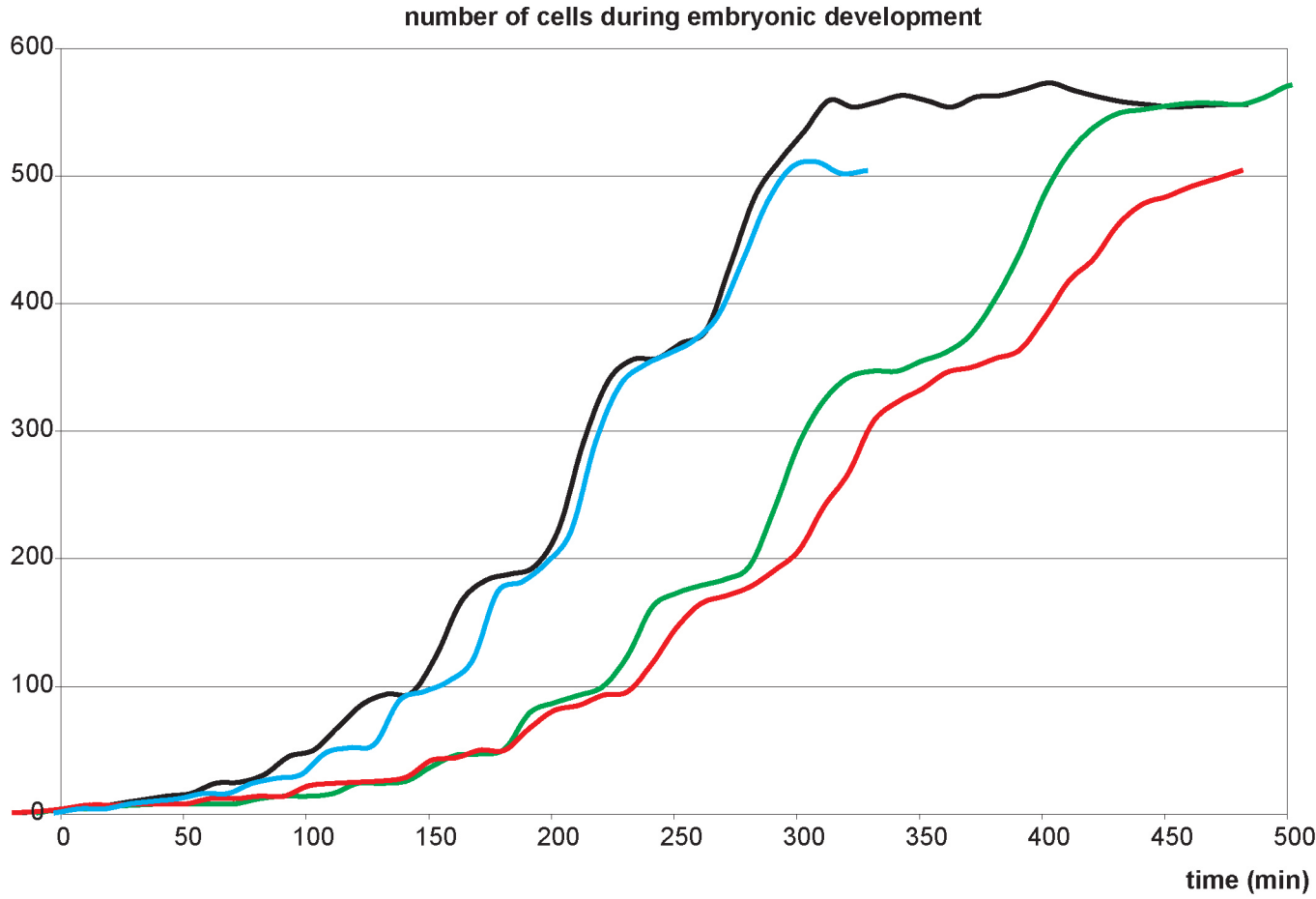
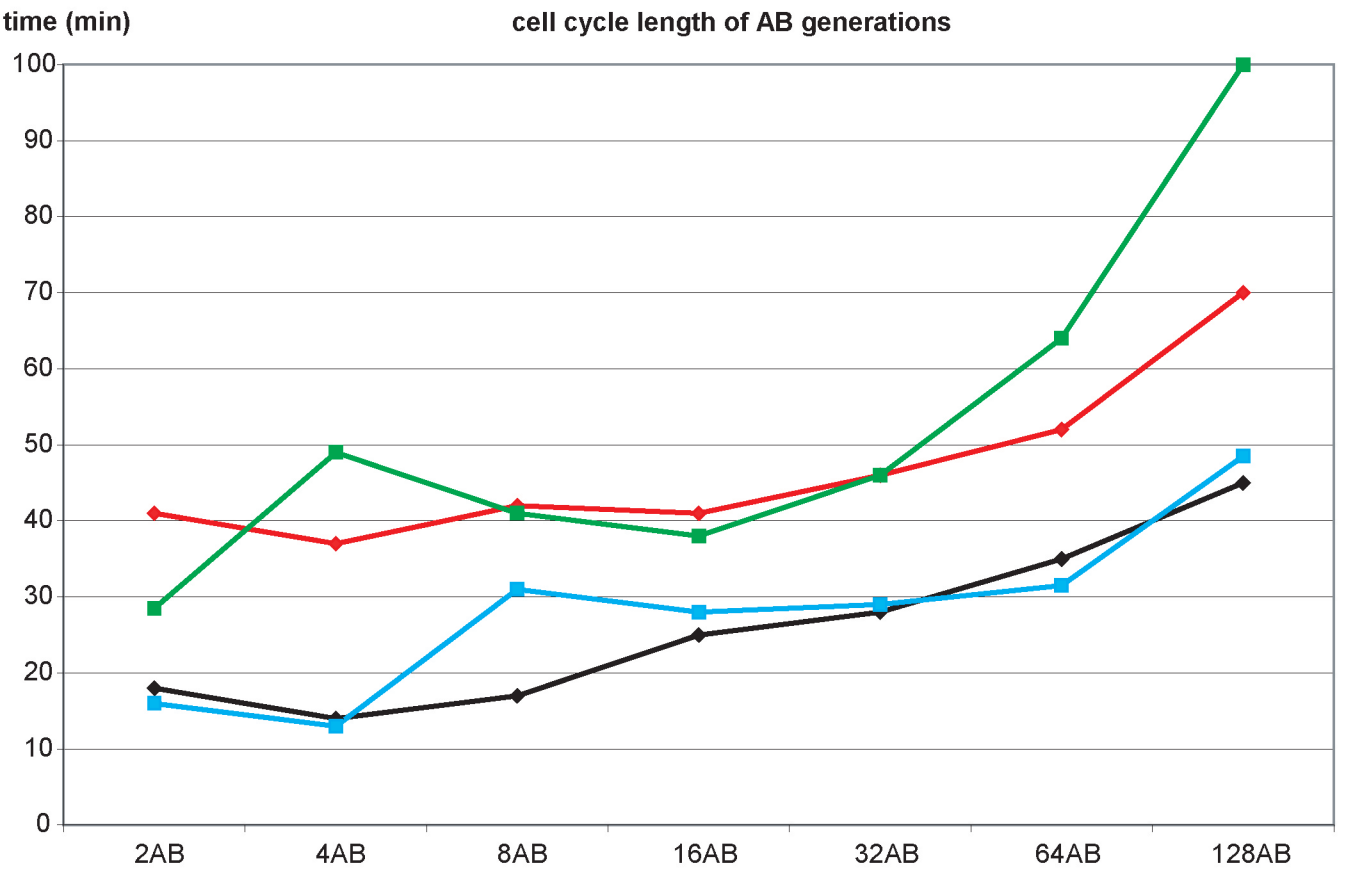
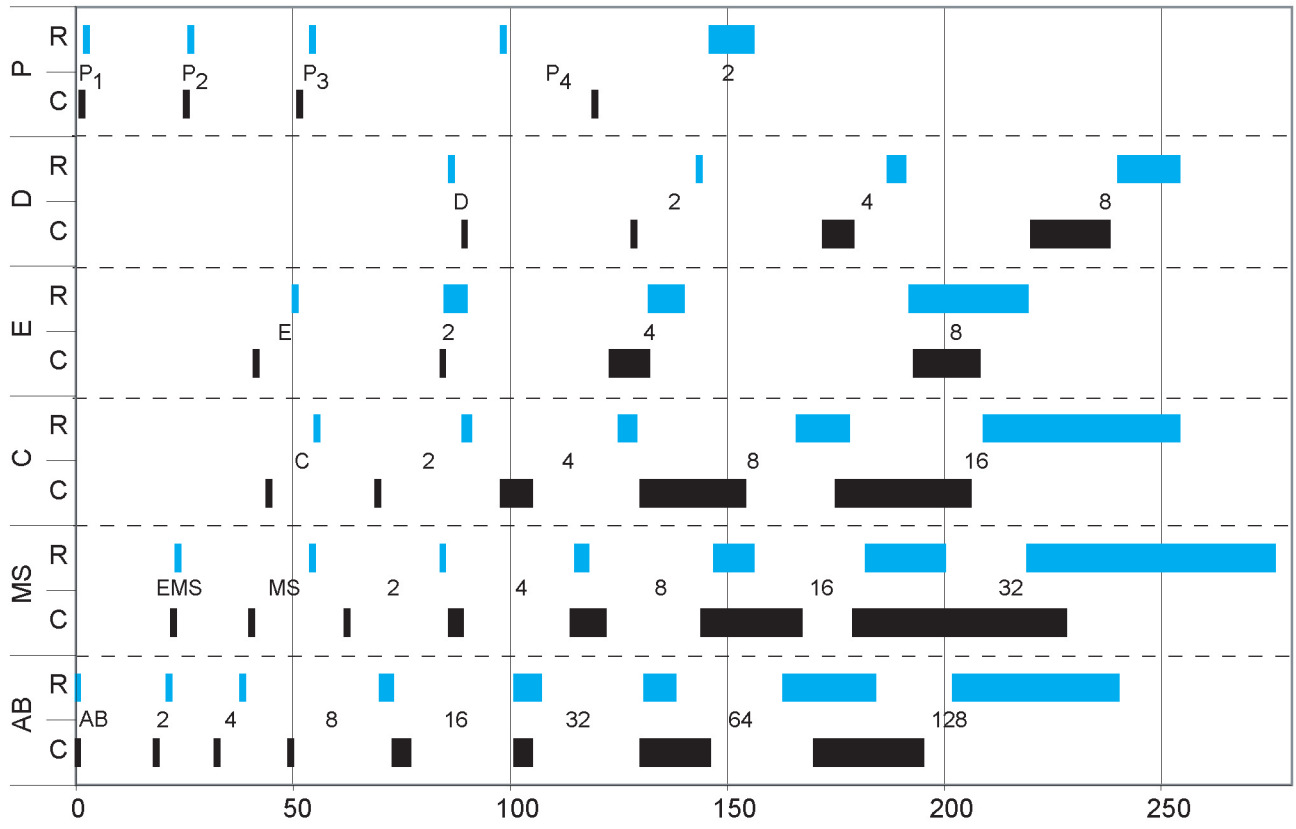

G


H


number of cells during embryonic development
600
500
400
300
200
100
0
0
50
100
150
200
250
300
350
400
450
500
time (min)


time (min)
cell cycle length of AB generations
100
90
80
70
60
50
40
30
20
10
0
2AB
4AB
8AB
16AB
32AB
64AB
128AB


P
D
E
C
MS
AB
R
C
P1
P2
P3
P4
2
D
2
4
8
E
2
4
8
C
2
4
8
16
EMS
MS
2
4
8
16
32
AB
2
4
8
16
32
64
128
0
50
100
150
200
250


P4
D
E
C
MS
AB
P
H
P1
P2
P3
P4
D
2
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8
E
2
4
8
C
2
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16
EMS
MS
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AB
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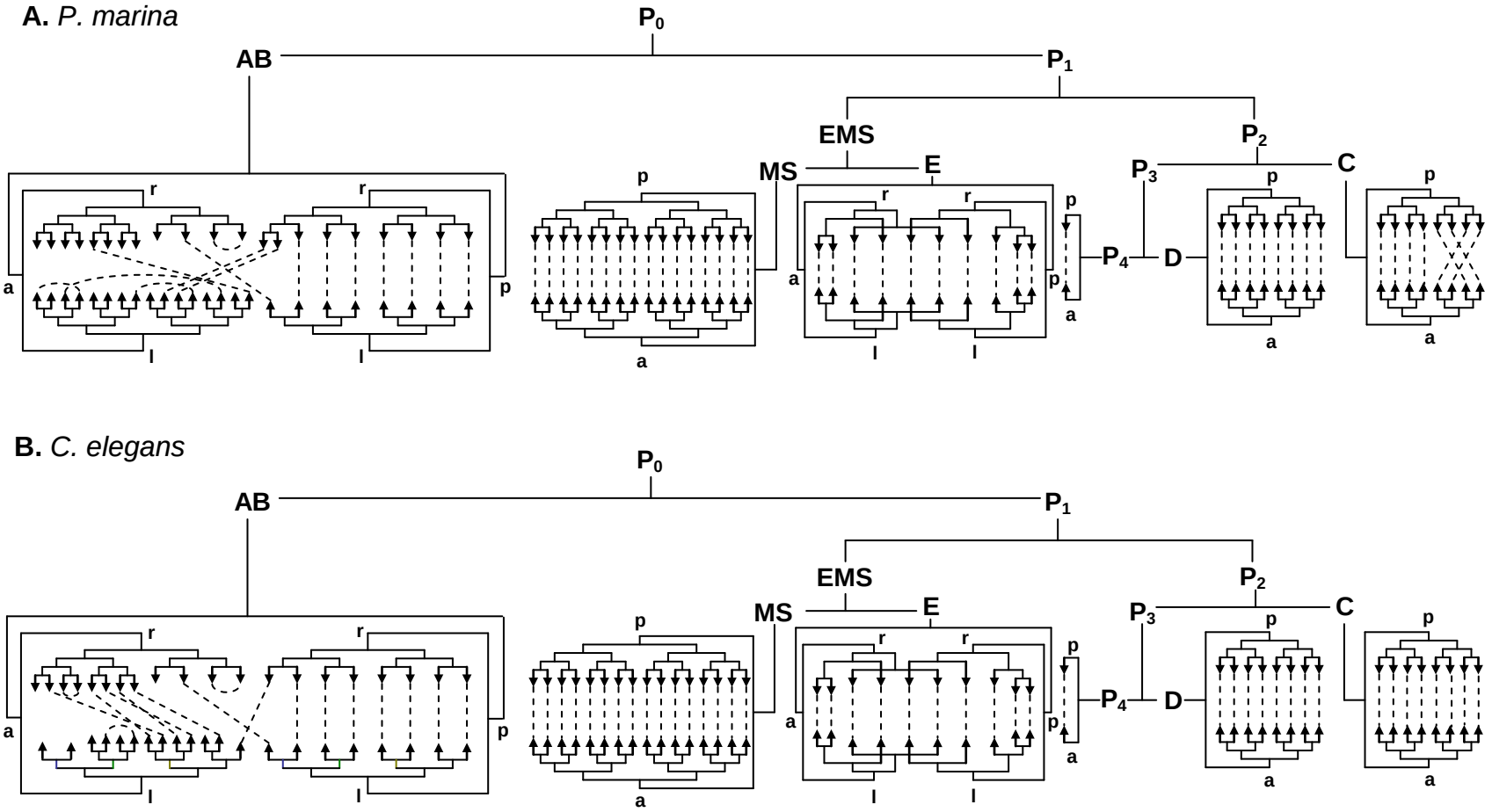
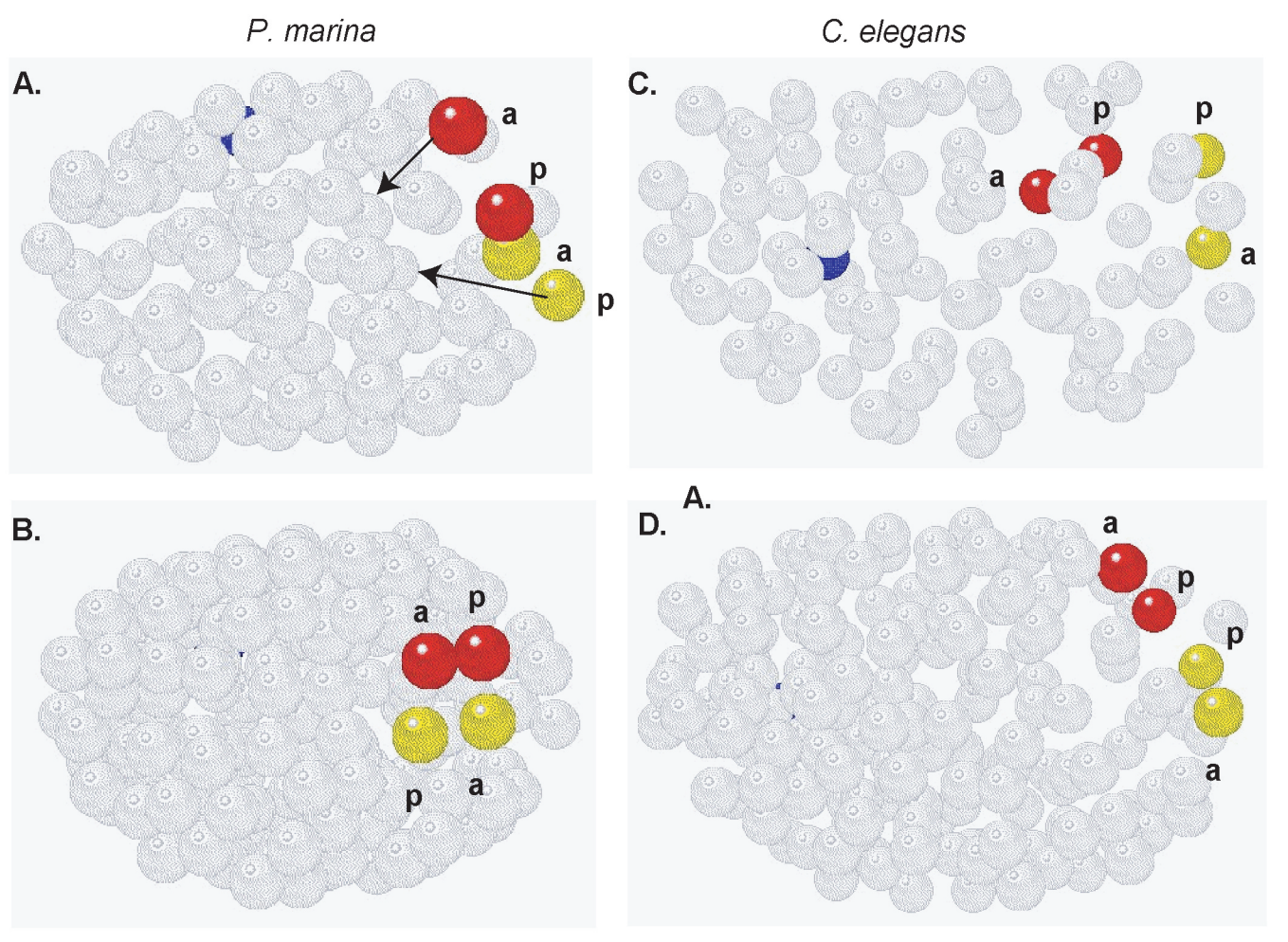
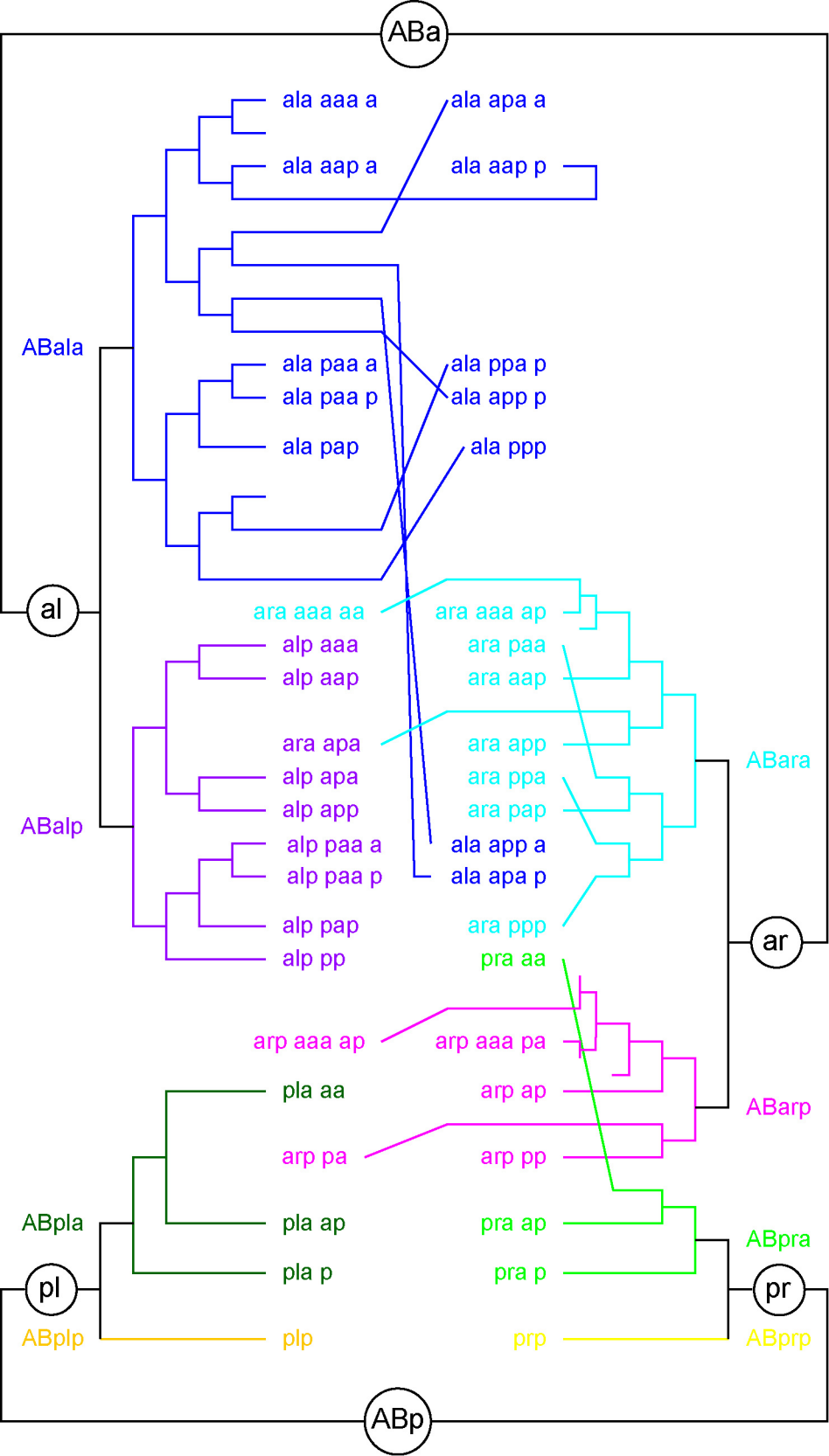

A. P. marina
P0
AB
P1
EMS
P2
MS
E
P3
C
P4
D
r
r
a
p
l
l
p
a
r
r
p
a
p
l
l
p
a
p
a
B. C. elegans
P0
AB
P1
EMS
P2
MS
E
P3
C
P4
D
r
r
a
p
l
l
p
a
r
r
p
a
p
l
l
p
a
p
a


P. marina
C. elegans
A.
a
p
a
p
B.
a
p
p
a
C.
p
p
a
a
A.
D.
a
p
p
a


ABa
ala aaa a
ala apa a
ala aap a
ala aap p
ABala
ala paa a
ala ppa p
ala paa p
ala app p
ala pap
ala ppp
al
ara aaa aa
ara aaa ap
alp aaa
ara paa
alp aap
ara aap
ara apa
ara app
ABara
alp apa
ara ppa
ABalp
alp app
ara pap
alp paa a
ala app a
alp paa p
ala apa p
alp pap
ara ppp
ar
alp pp
pra aa
arp aaa ap
arp aaa pa
pla aa
arp ap
ABarp
arp pa
arp pp
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pra ap
ABpra
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ABplp
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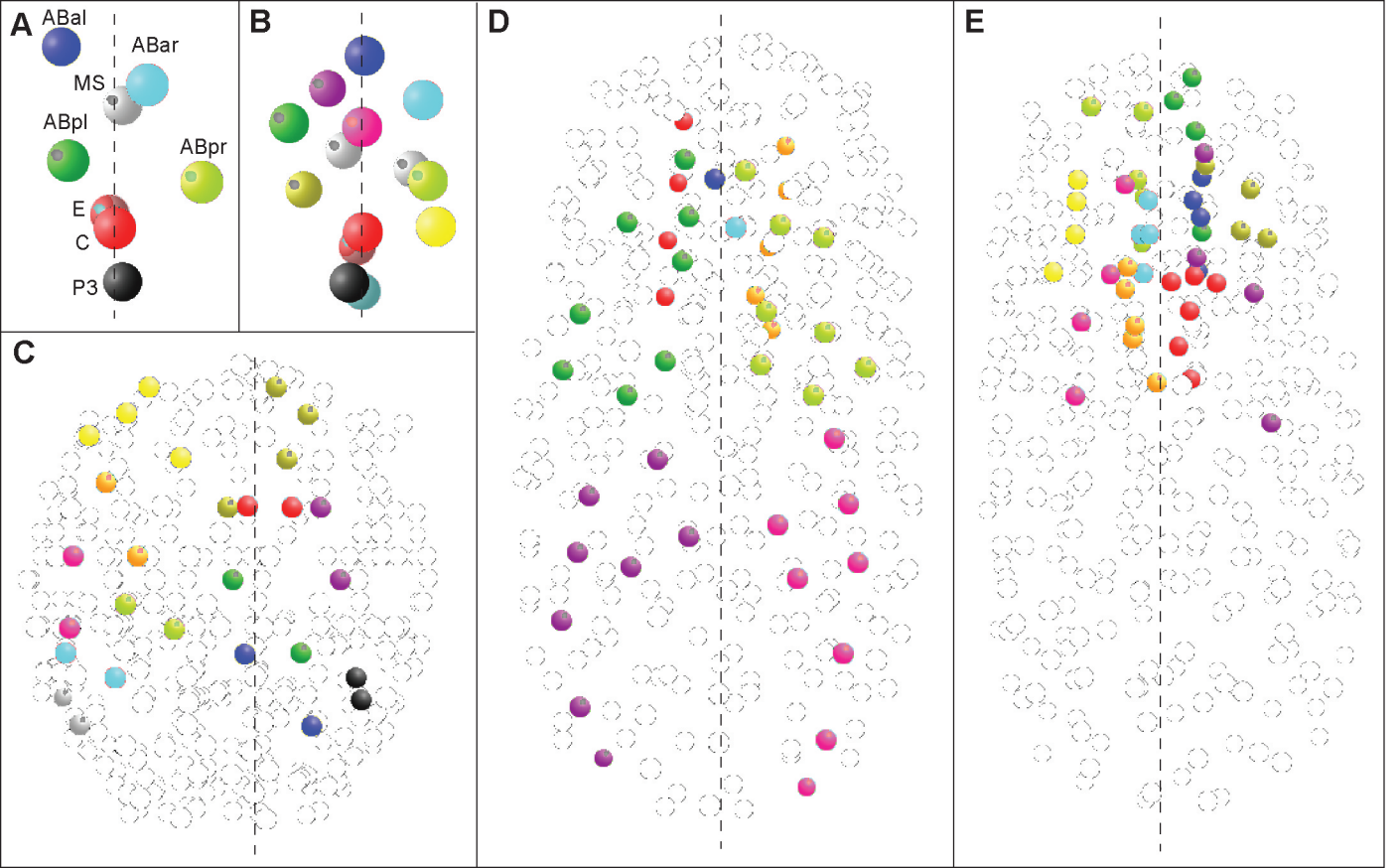
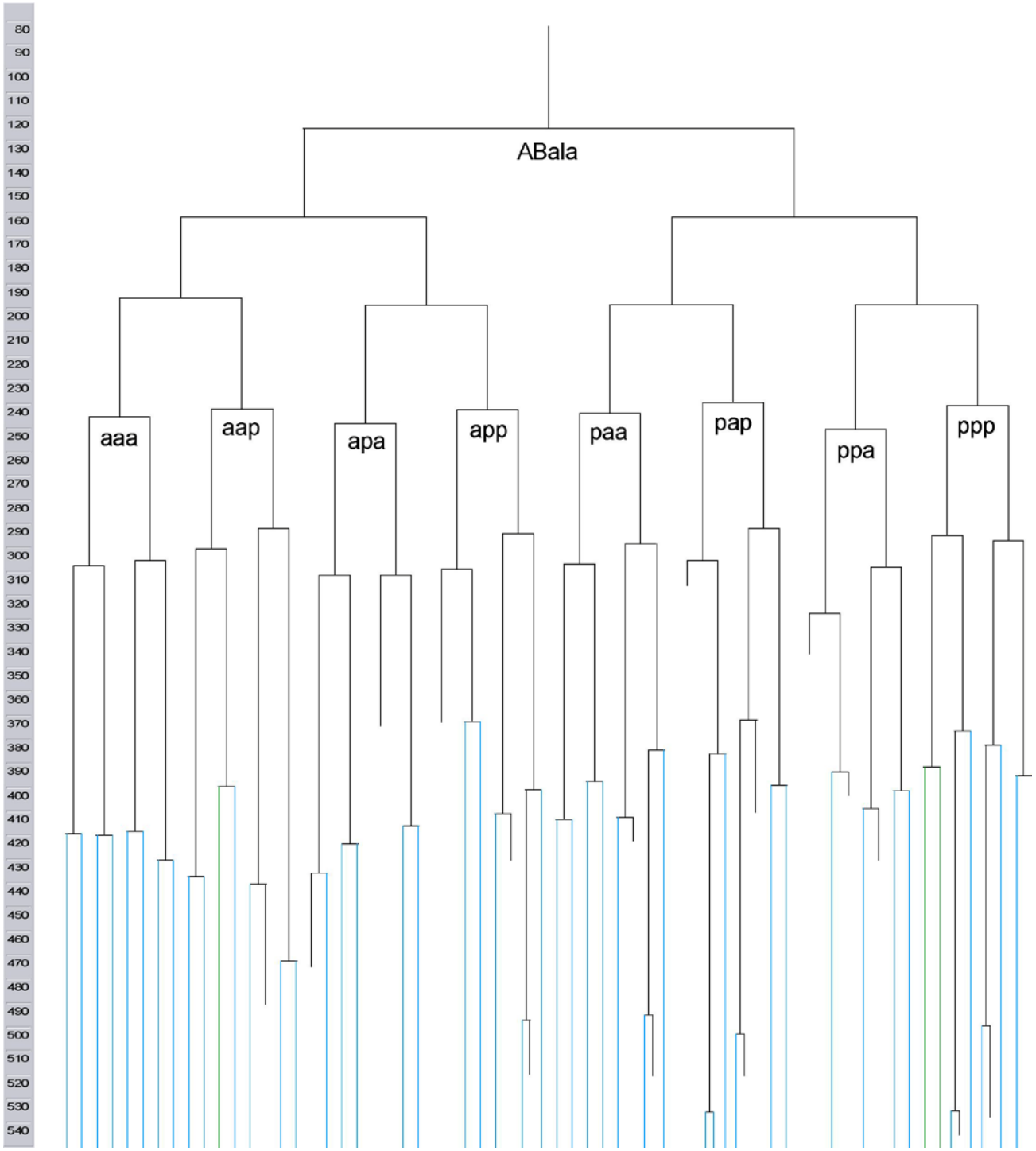
prp
ABprp
ABp


A
ABal
ABar
MS
ABpl
ABpr
E
C
P3
B
C
D
E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ABala
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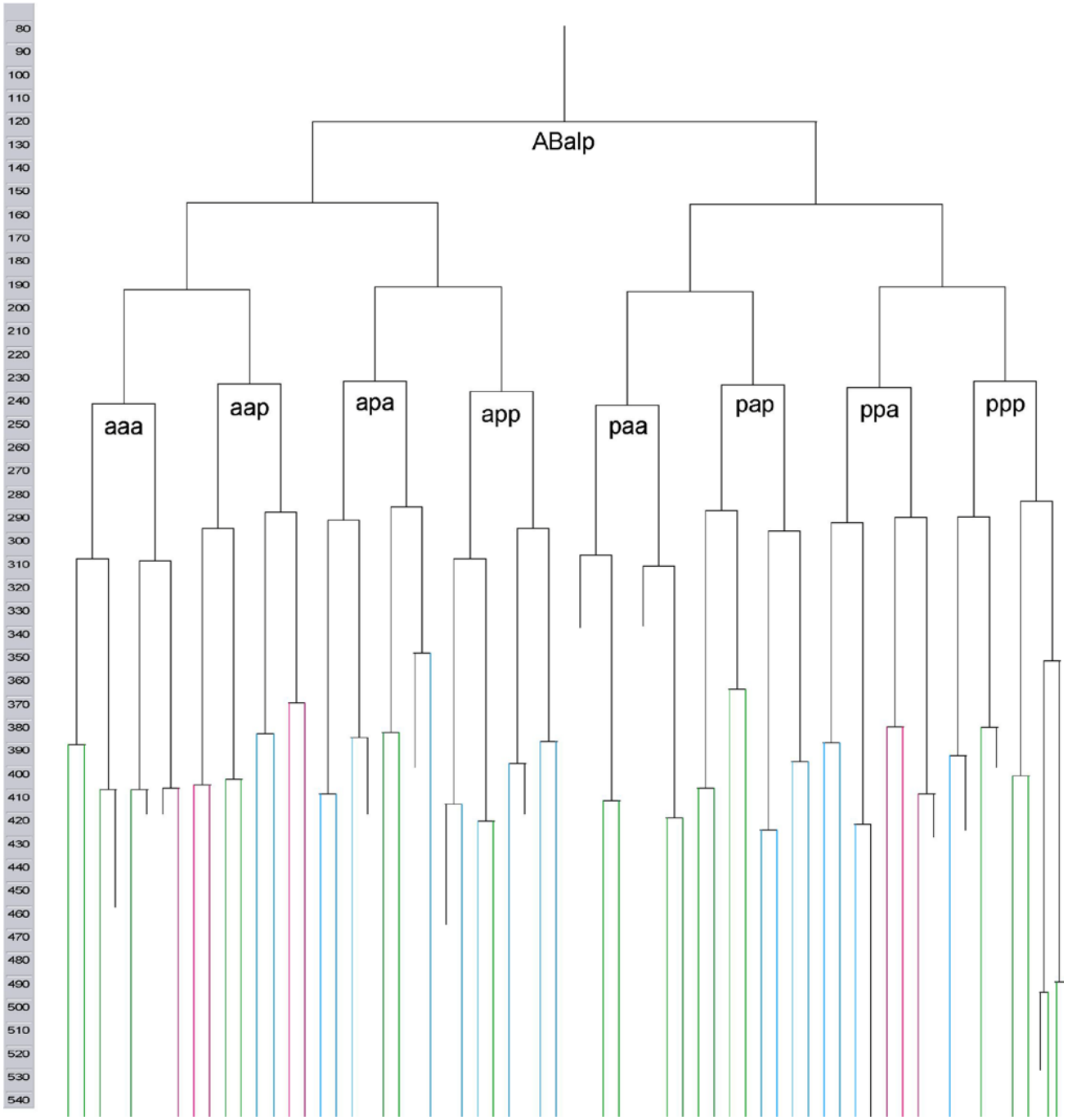
ppp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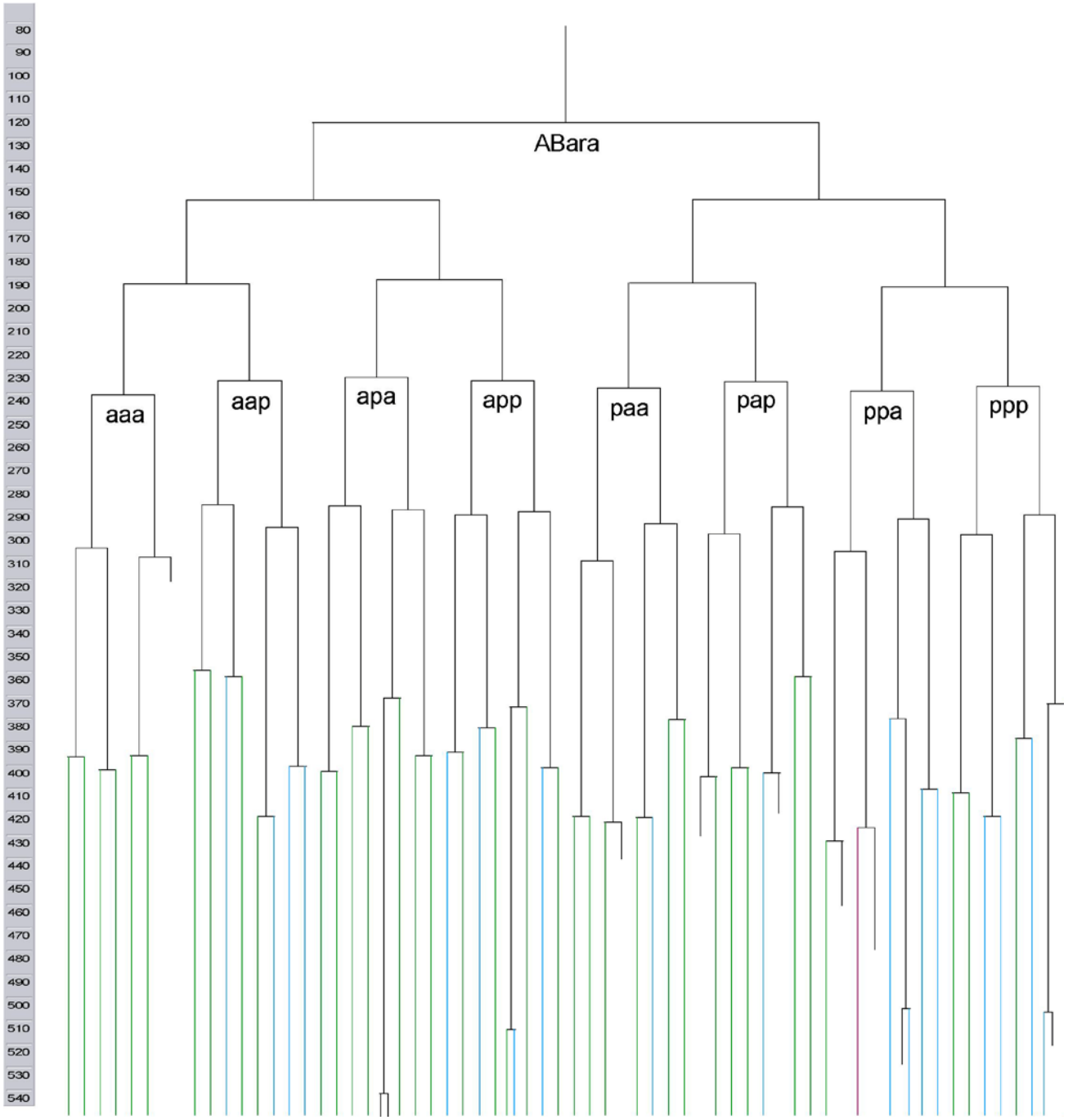
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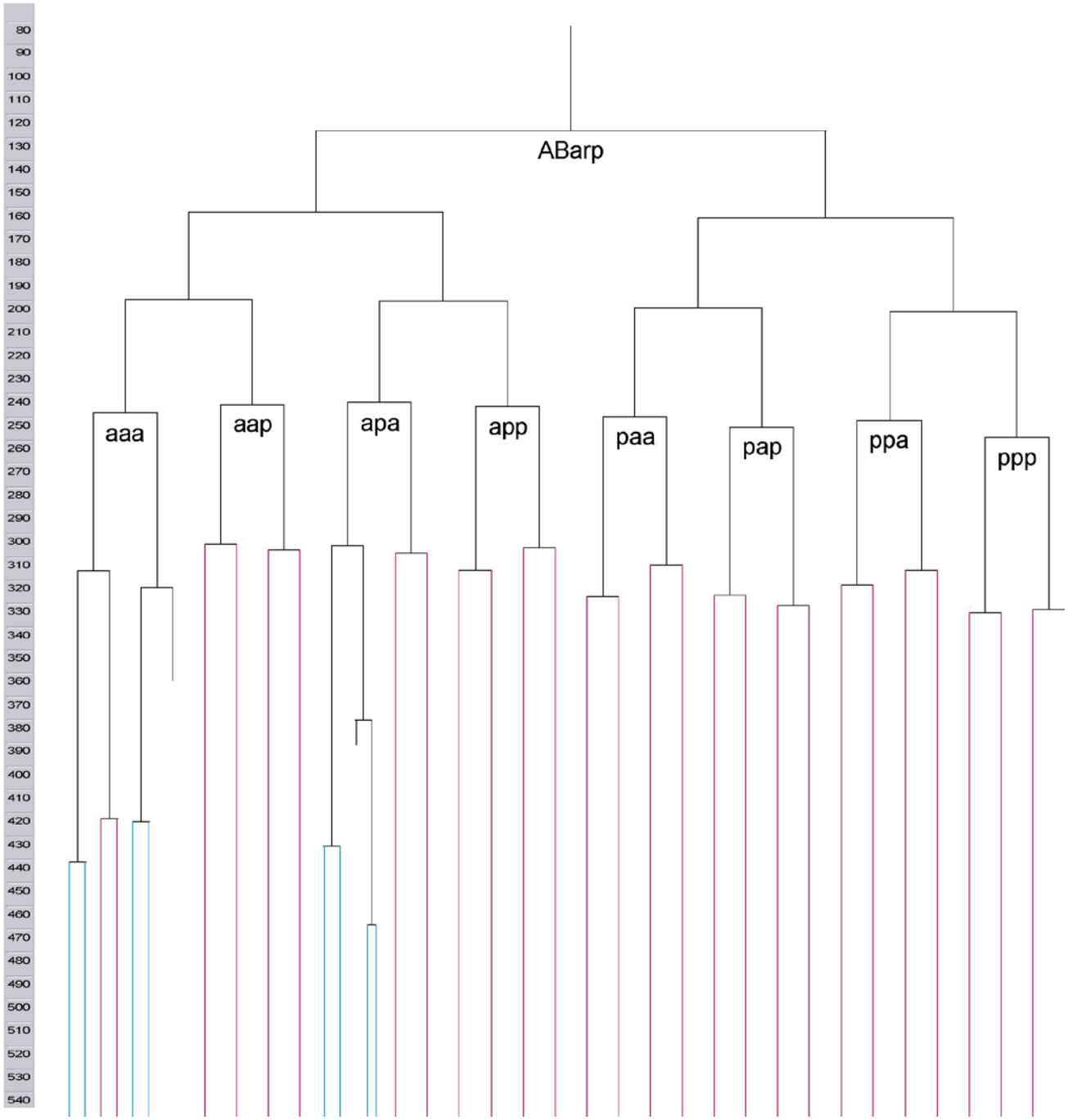
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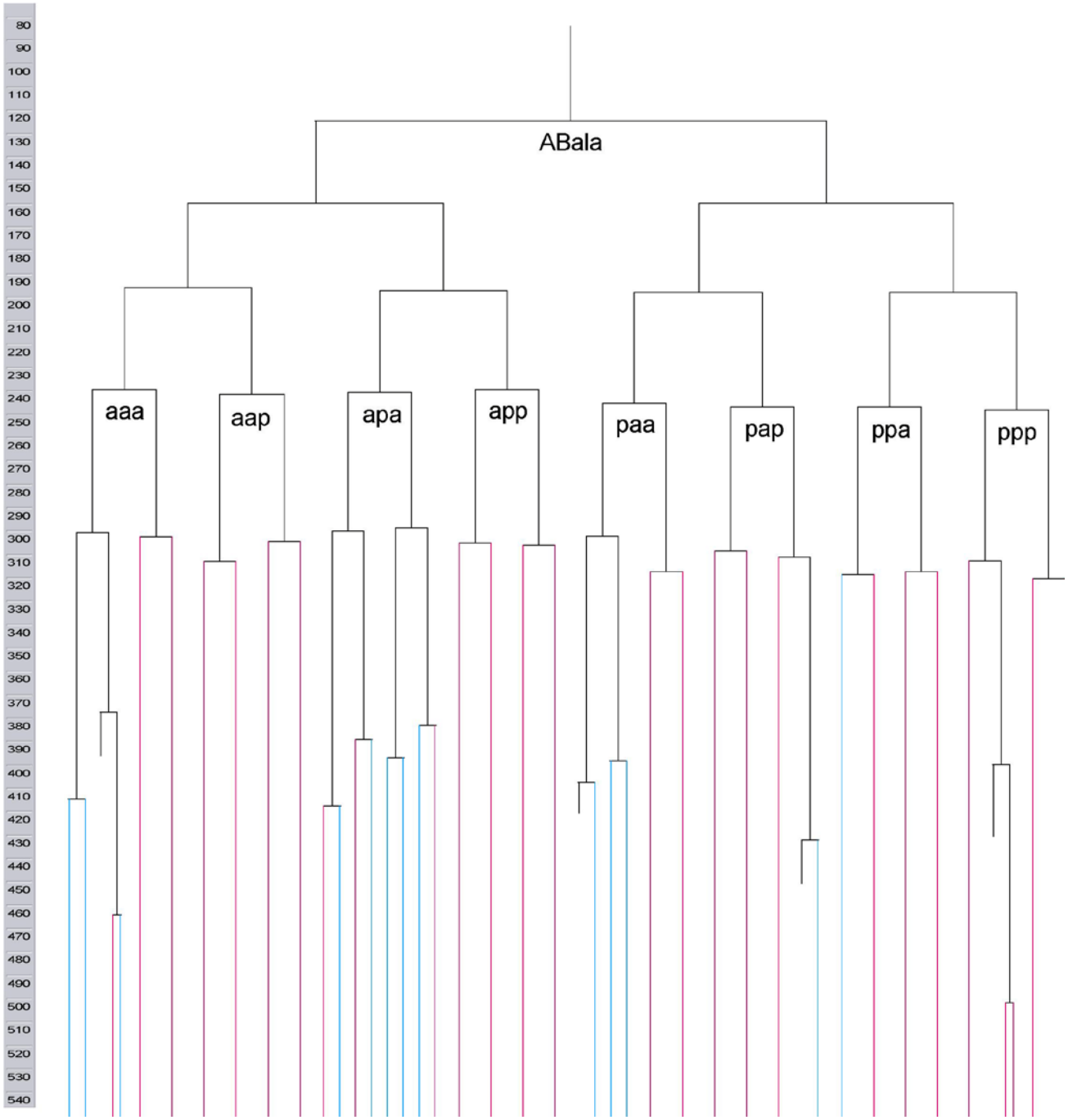
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ABala
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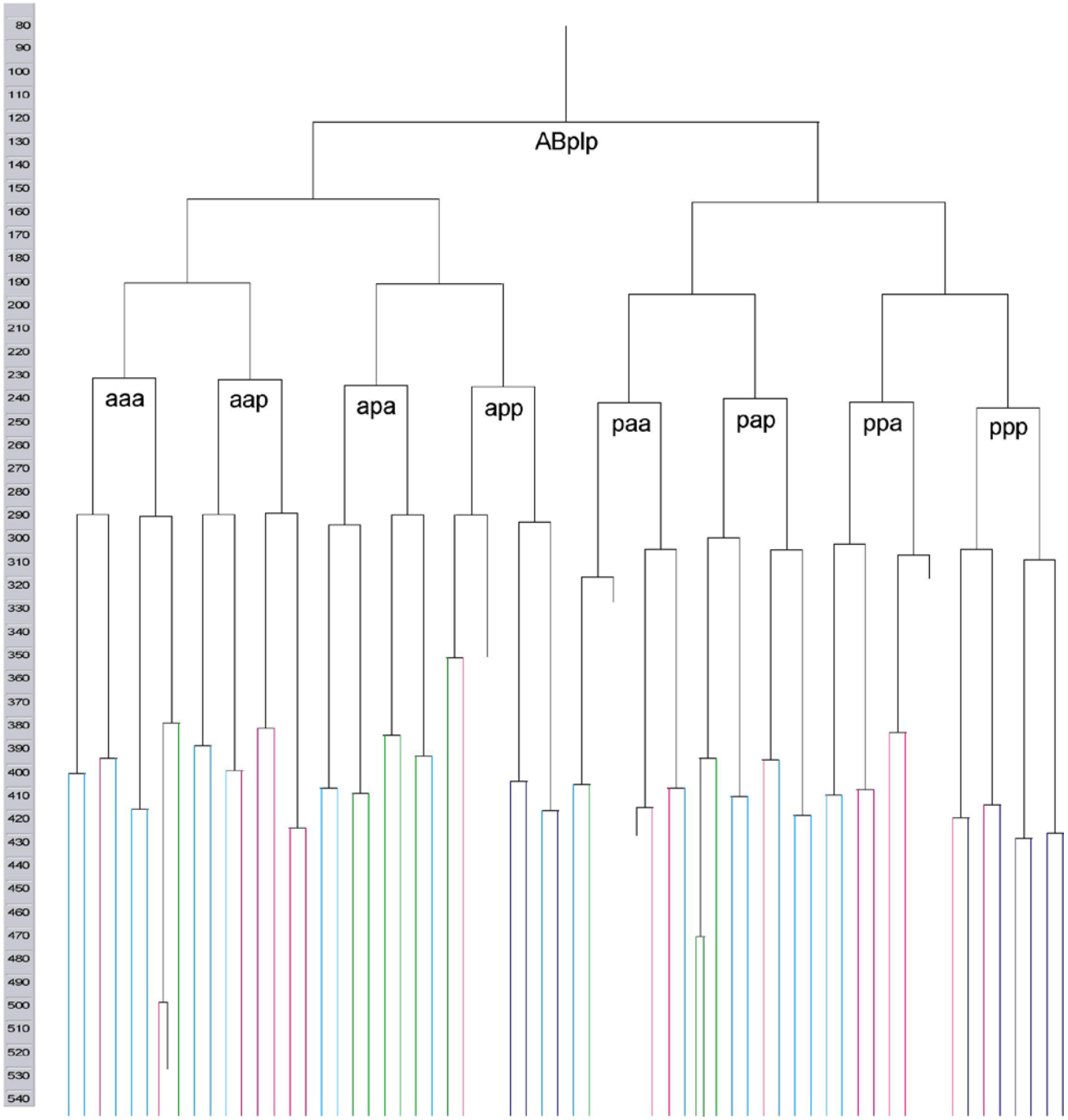
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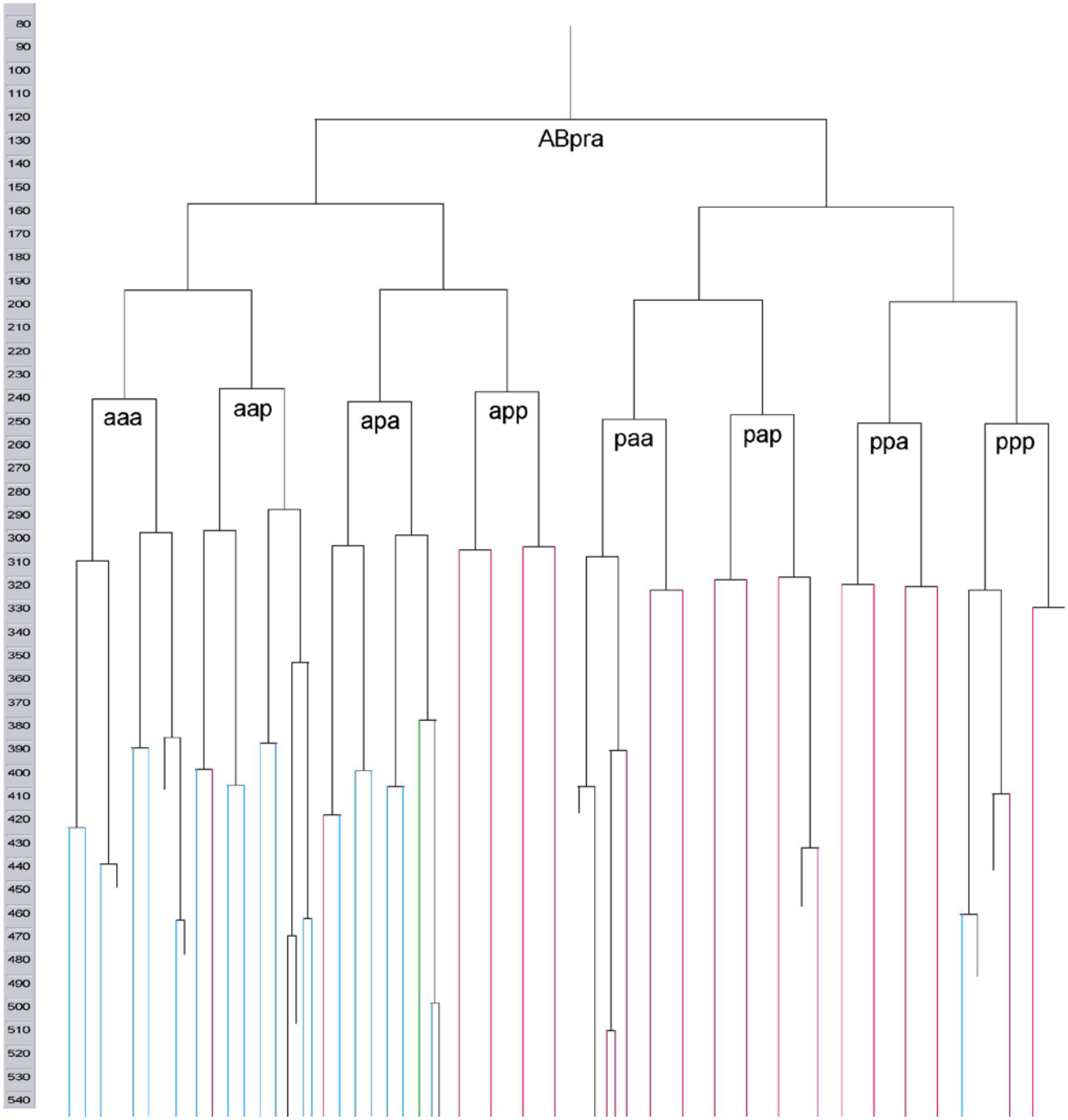
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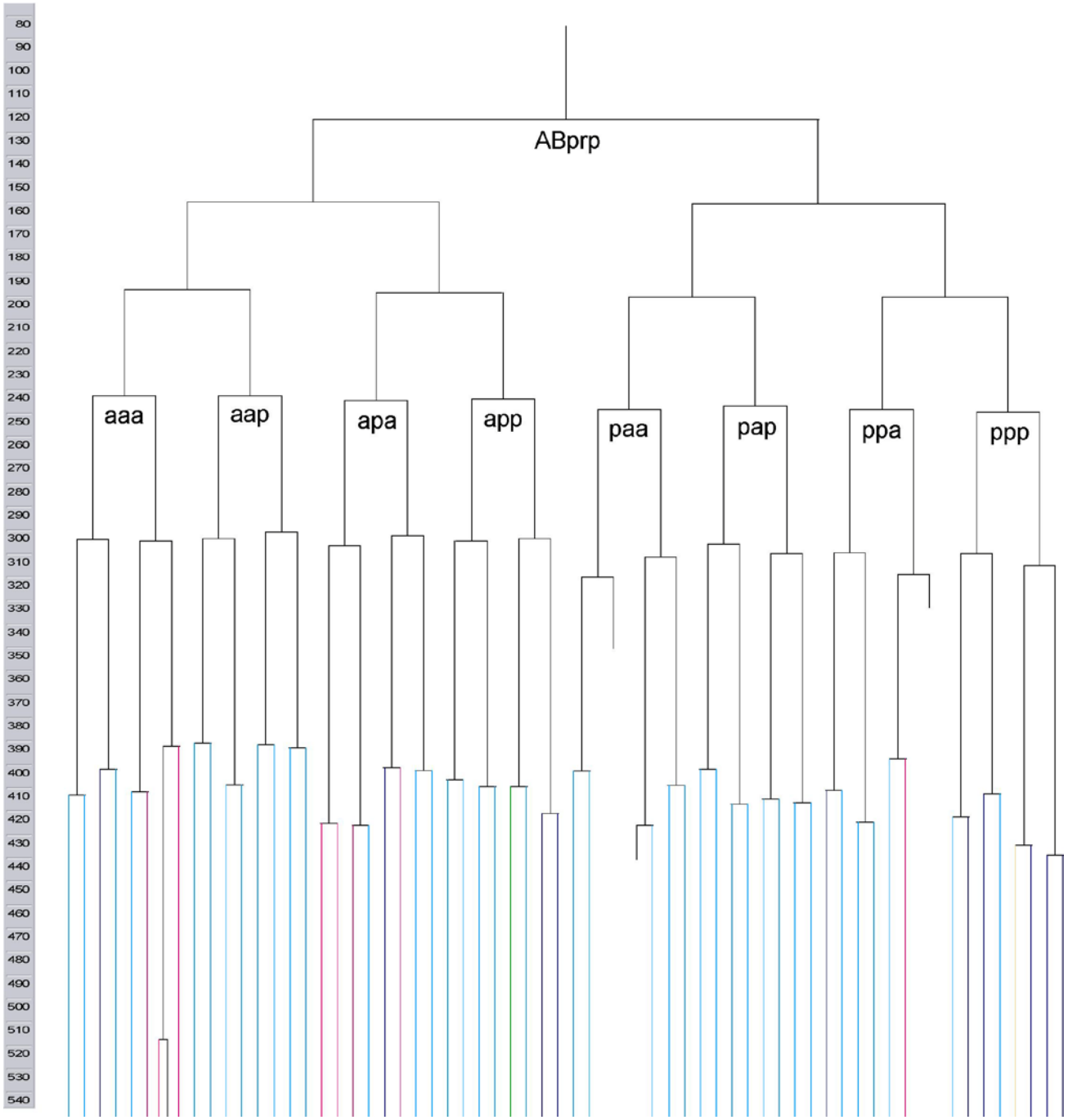
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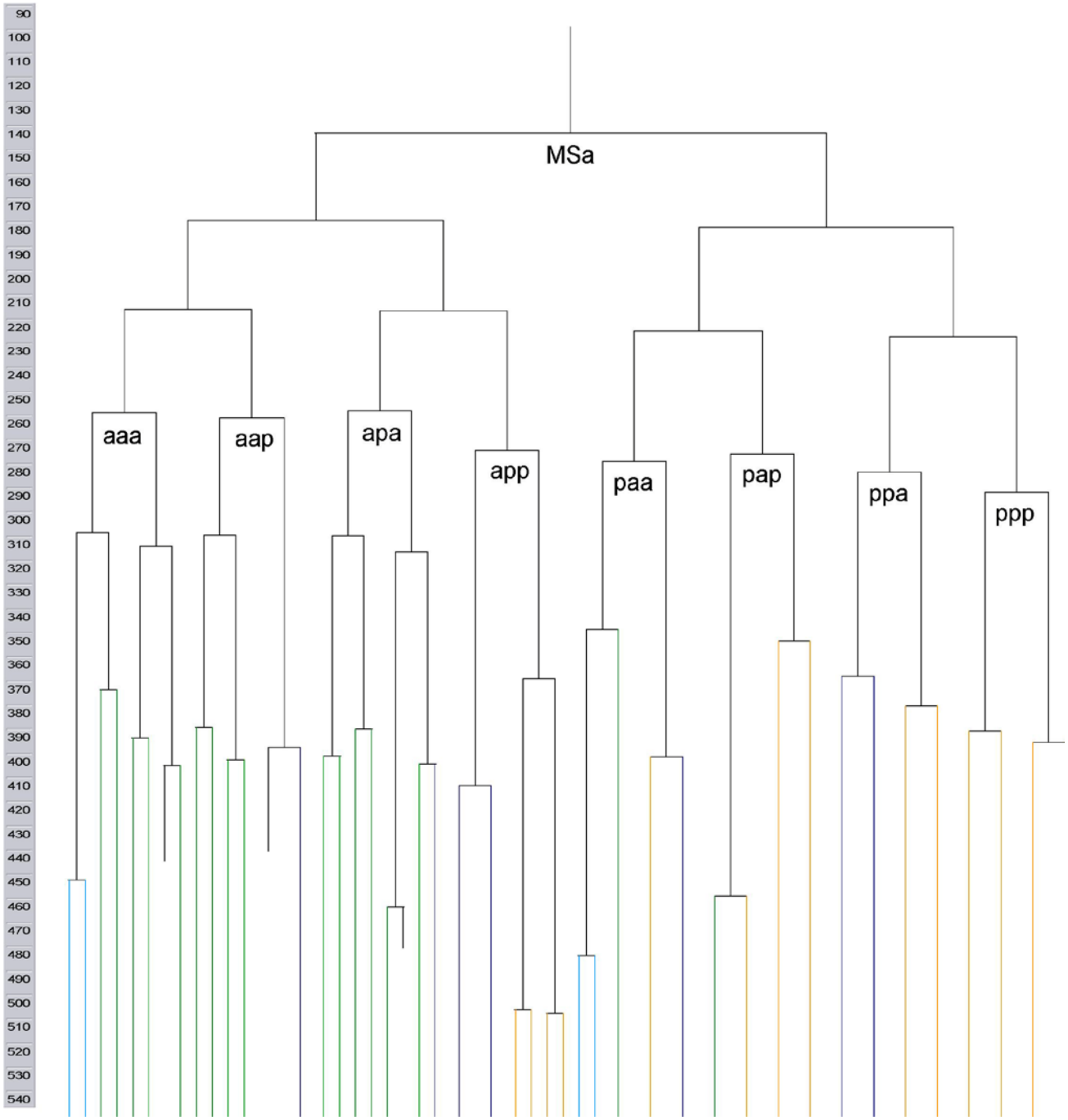
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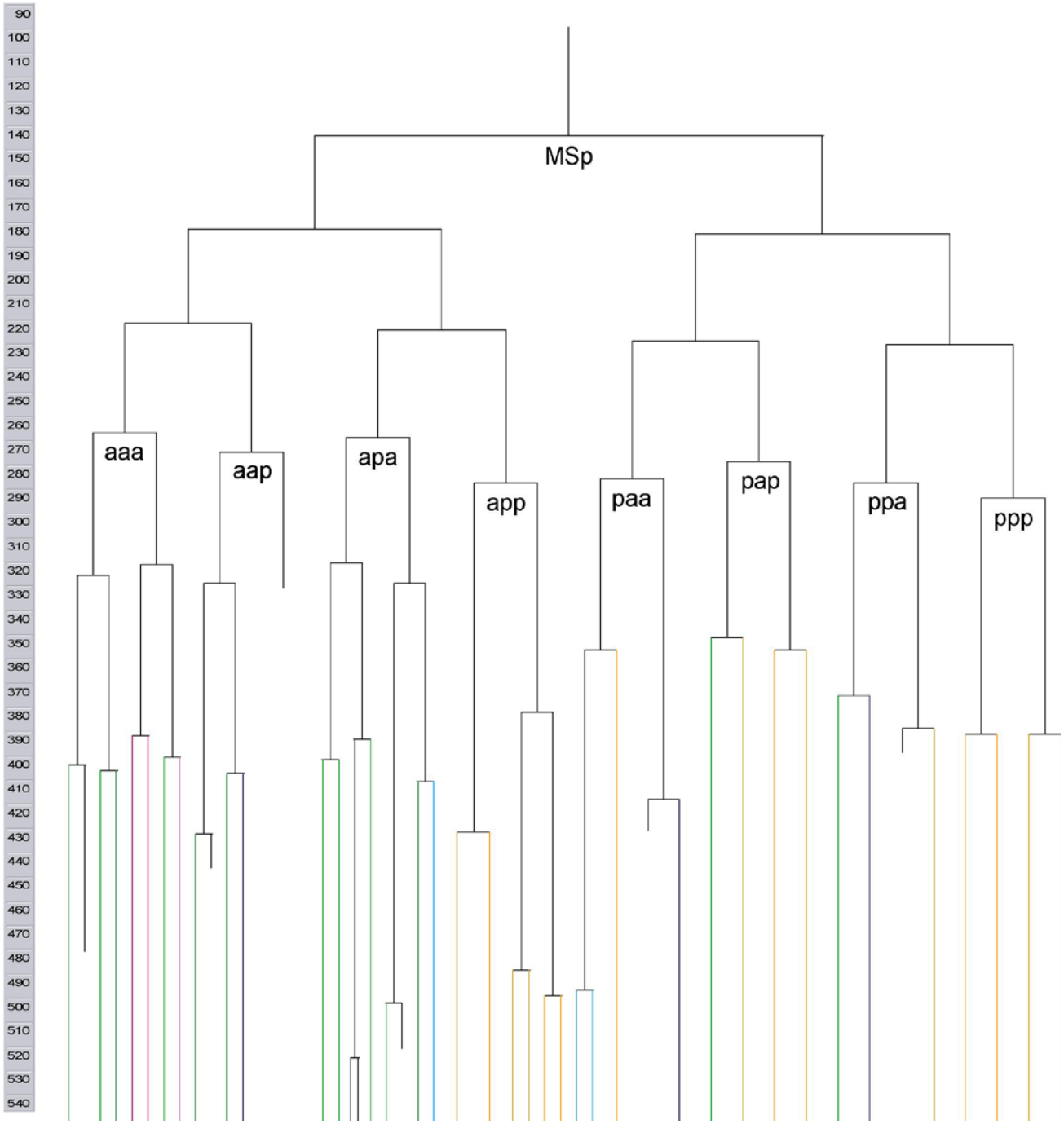
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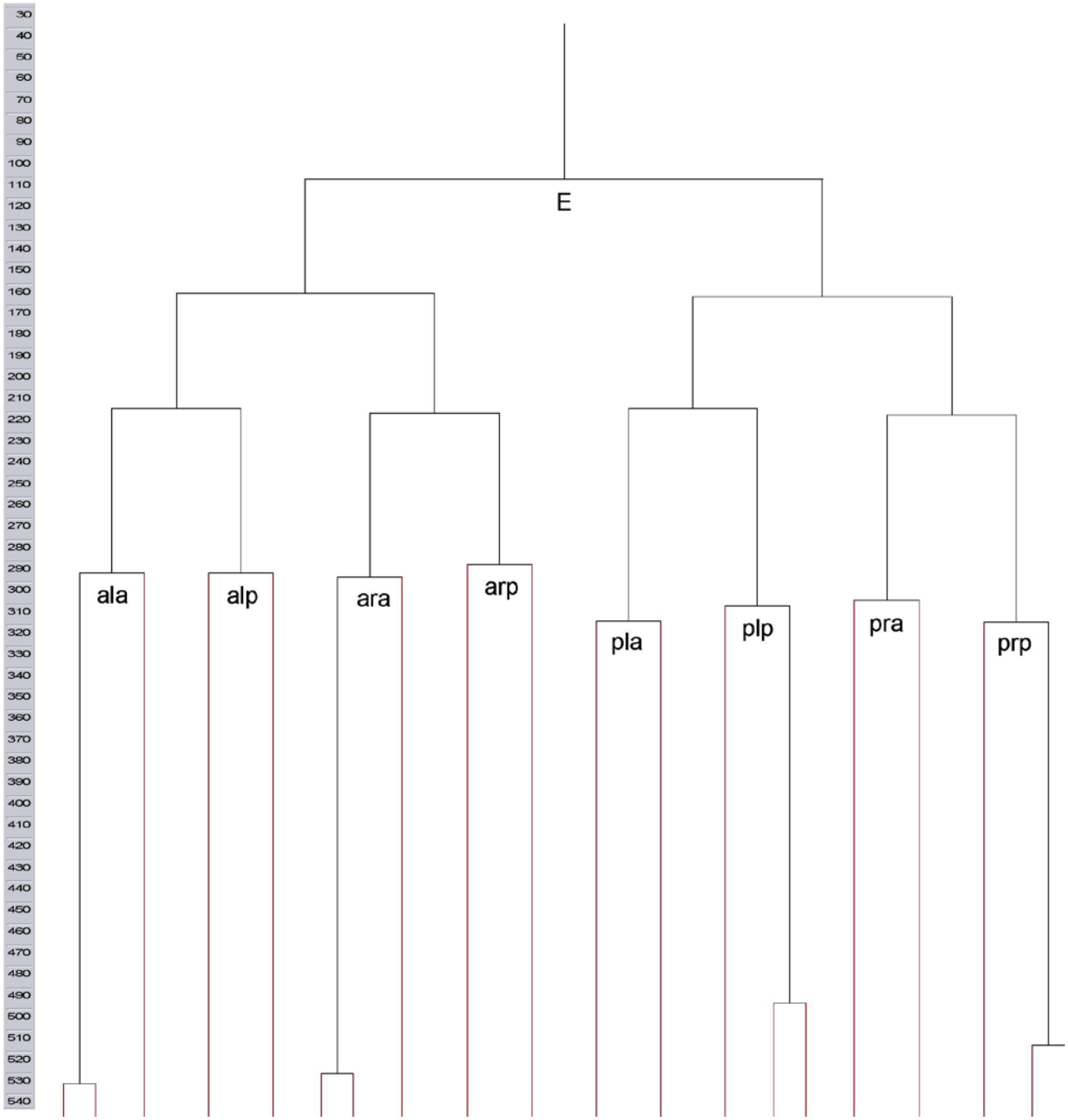
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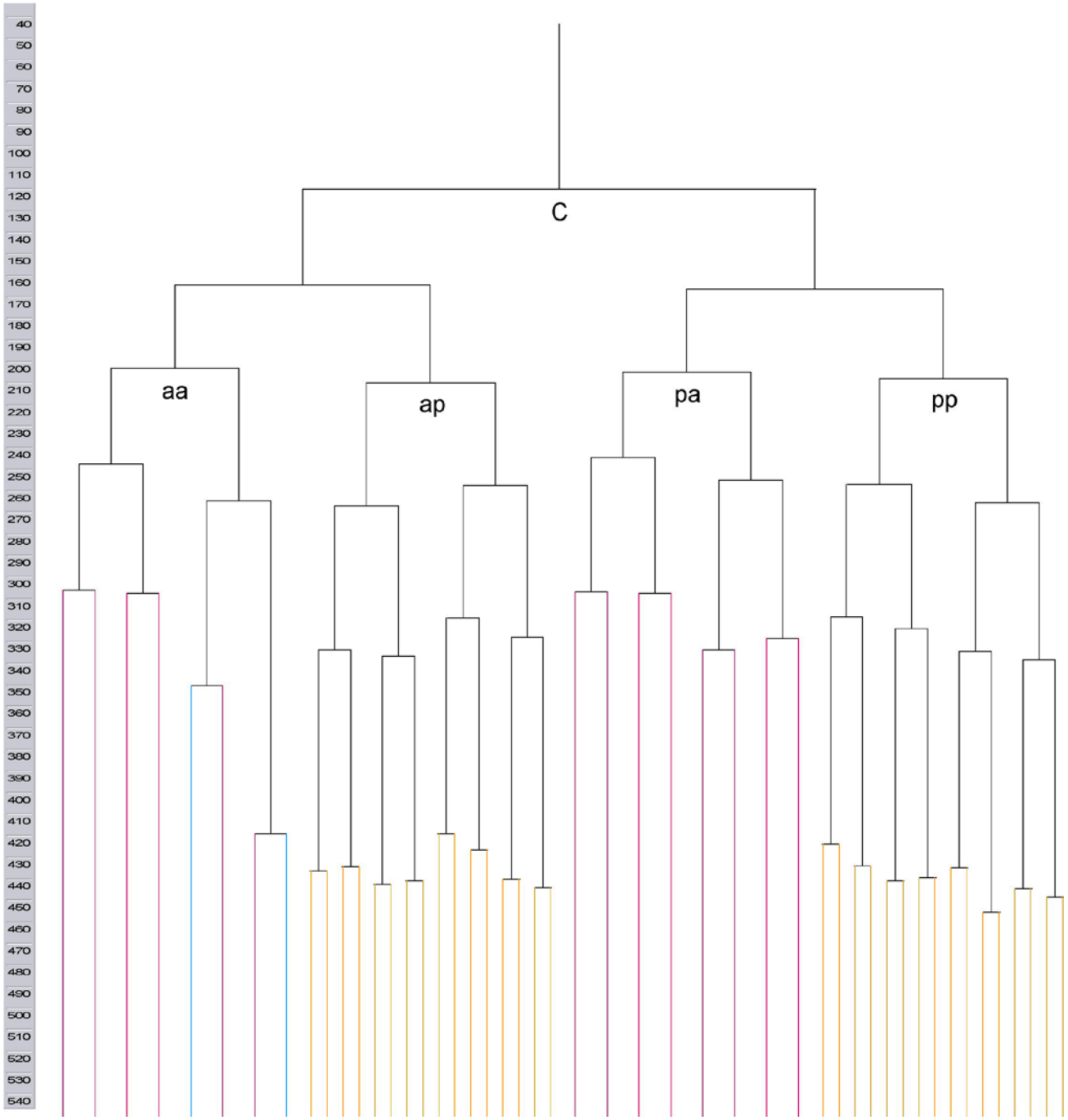
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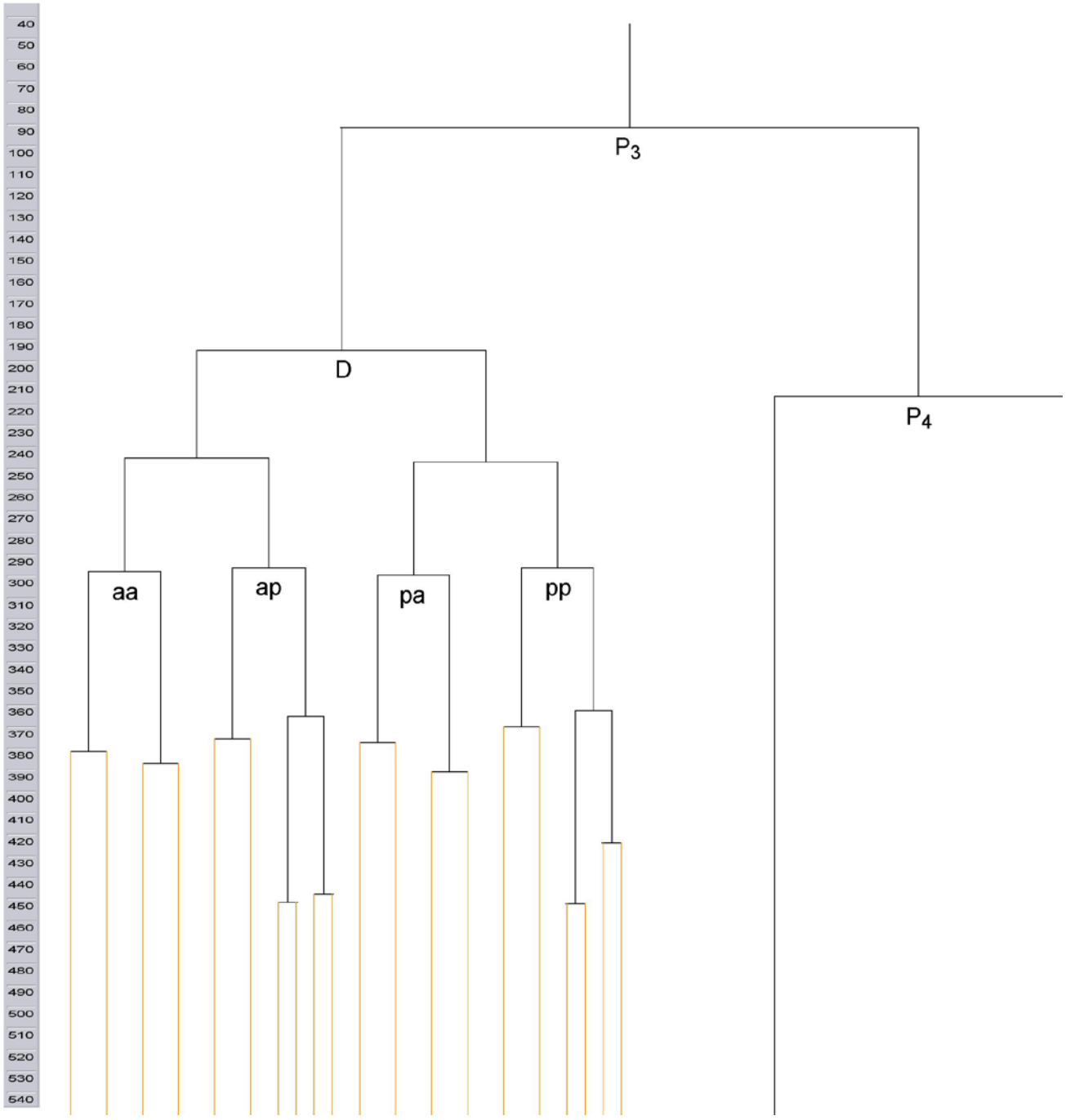
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MSappaap
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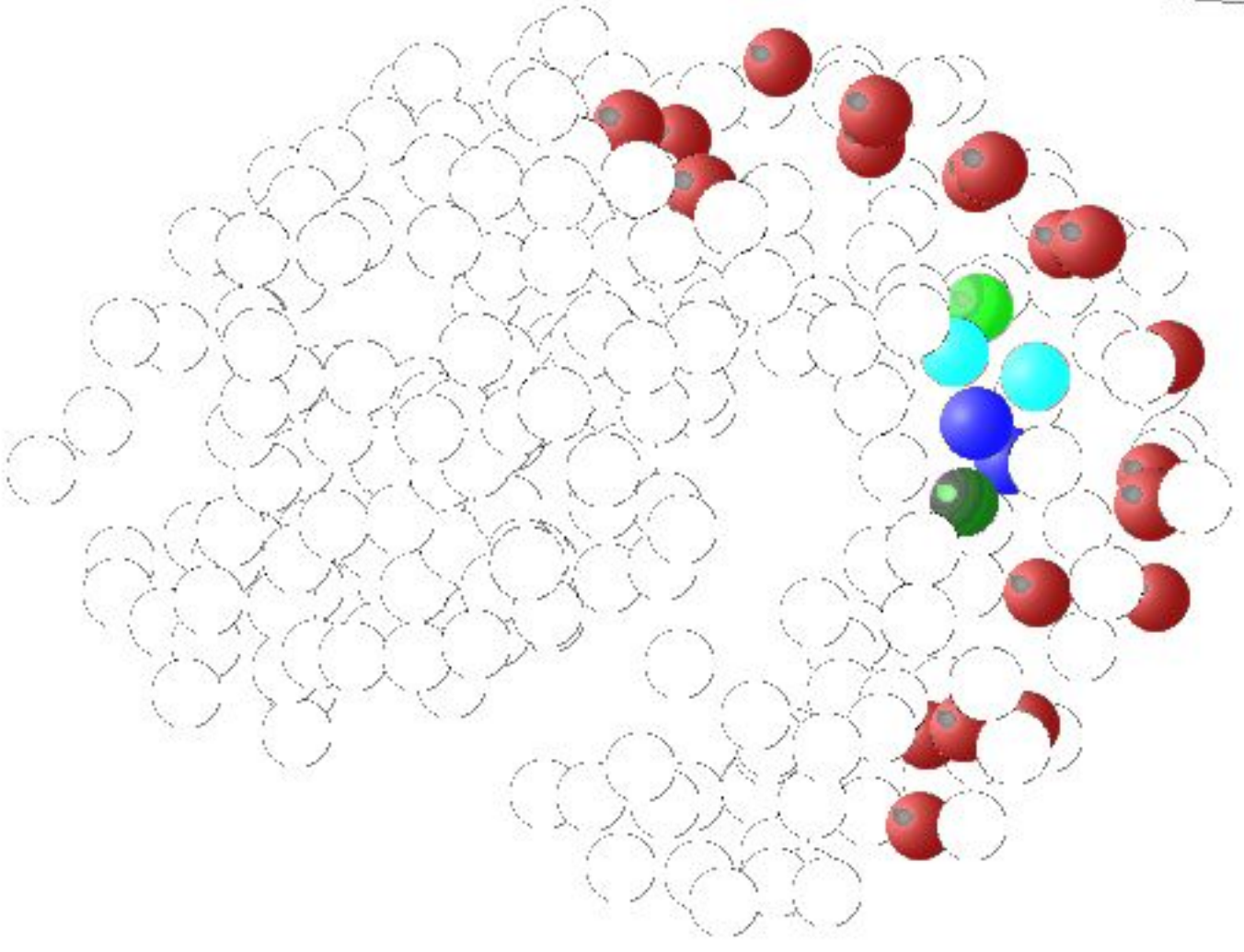
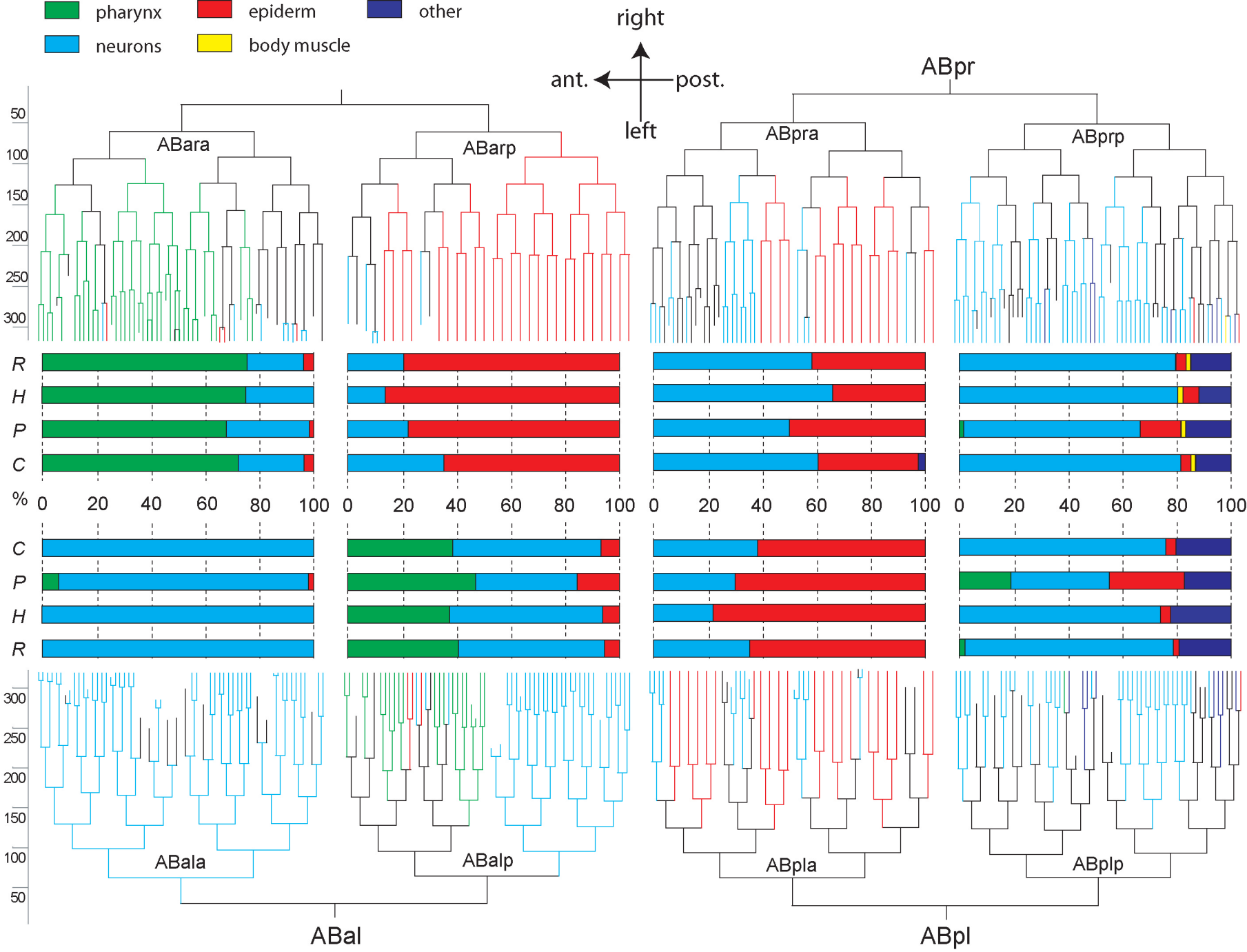
MSpppaap




pharynx
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body muscle
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ABpr
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ABala
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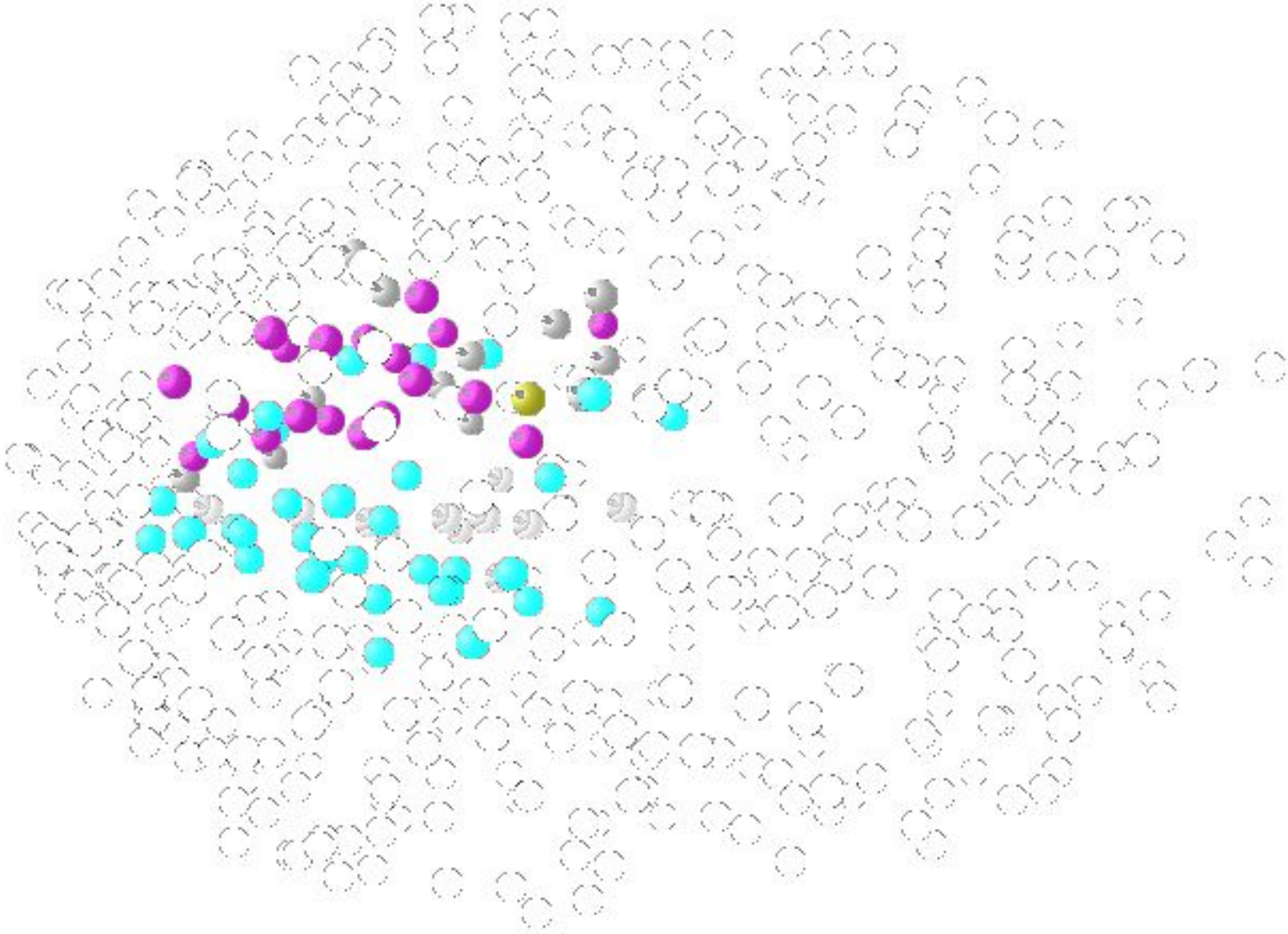

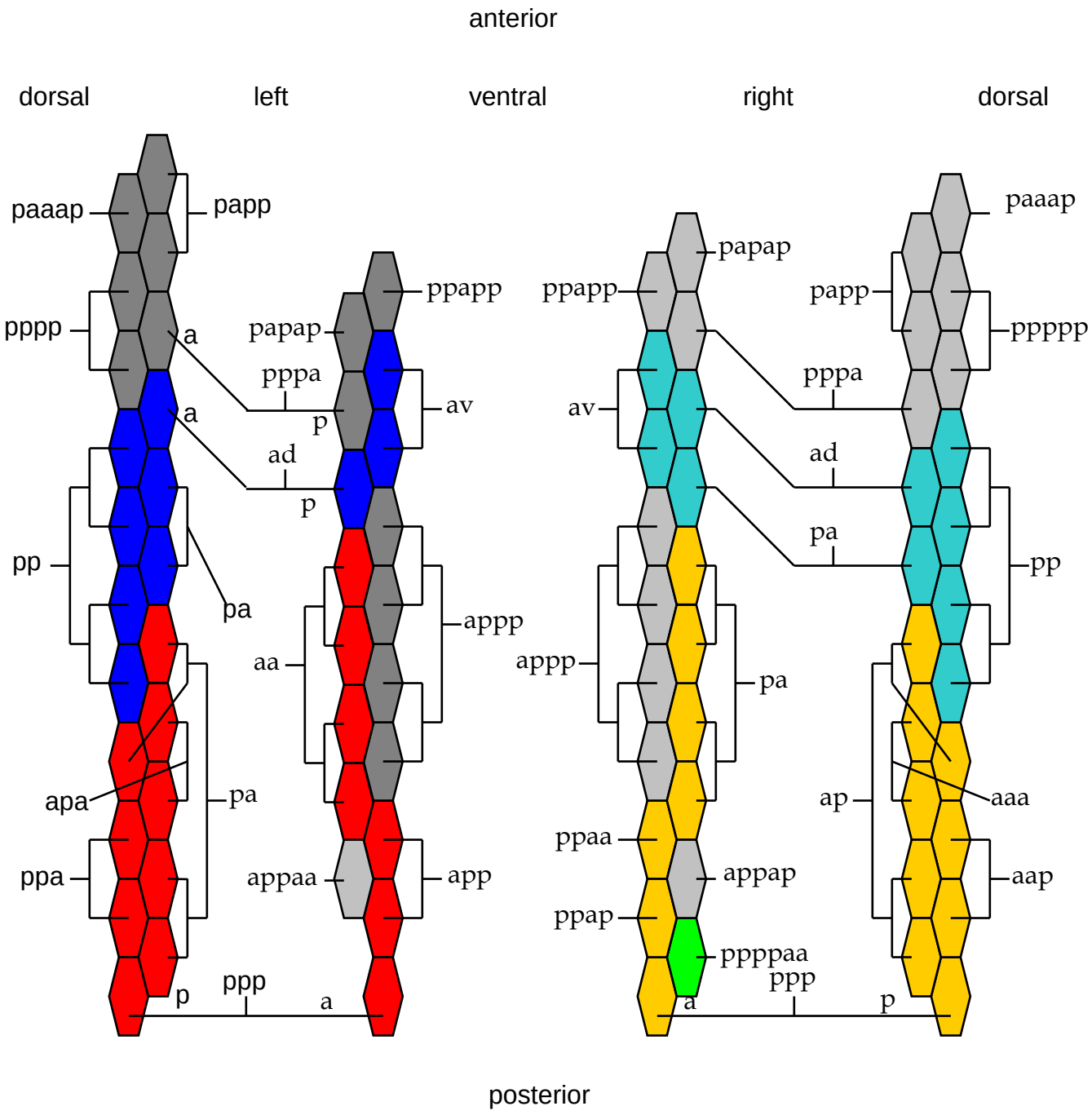
anterior
dorsal
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dorsal
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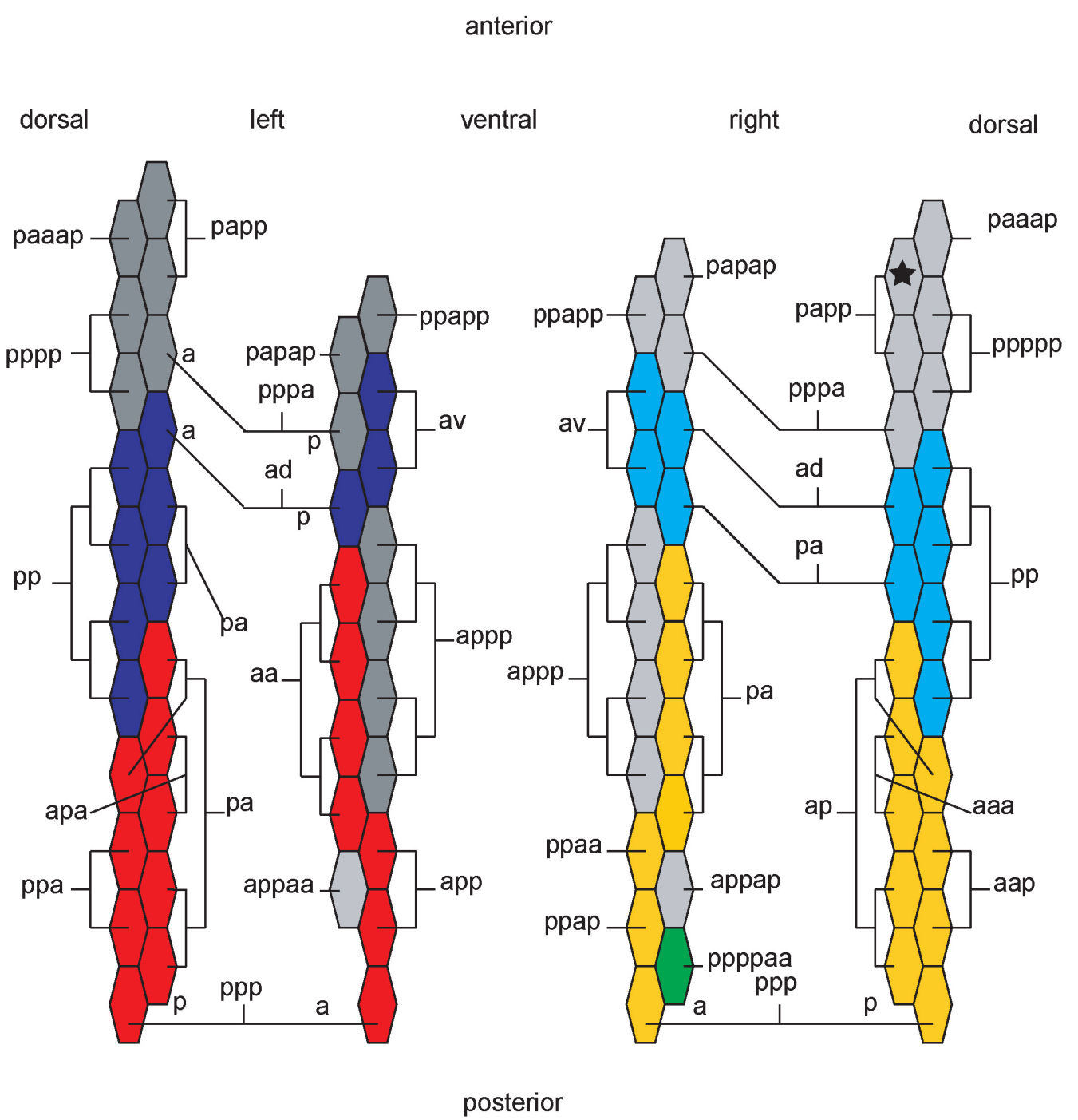
posterior


anterior
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appp
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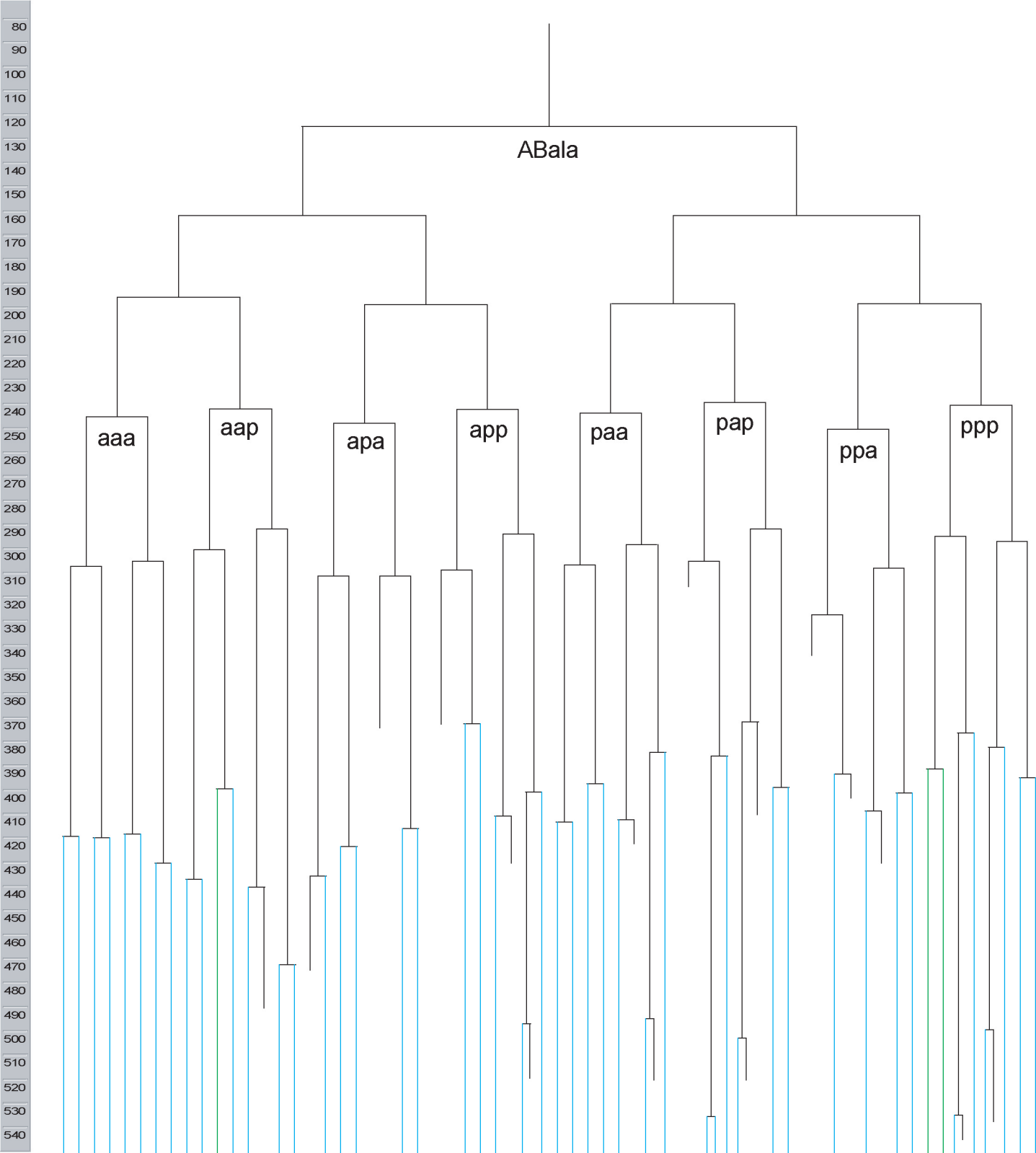
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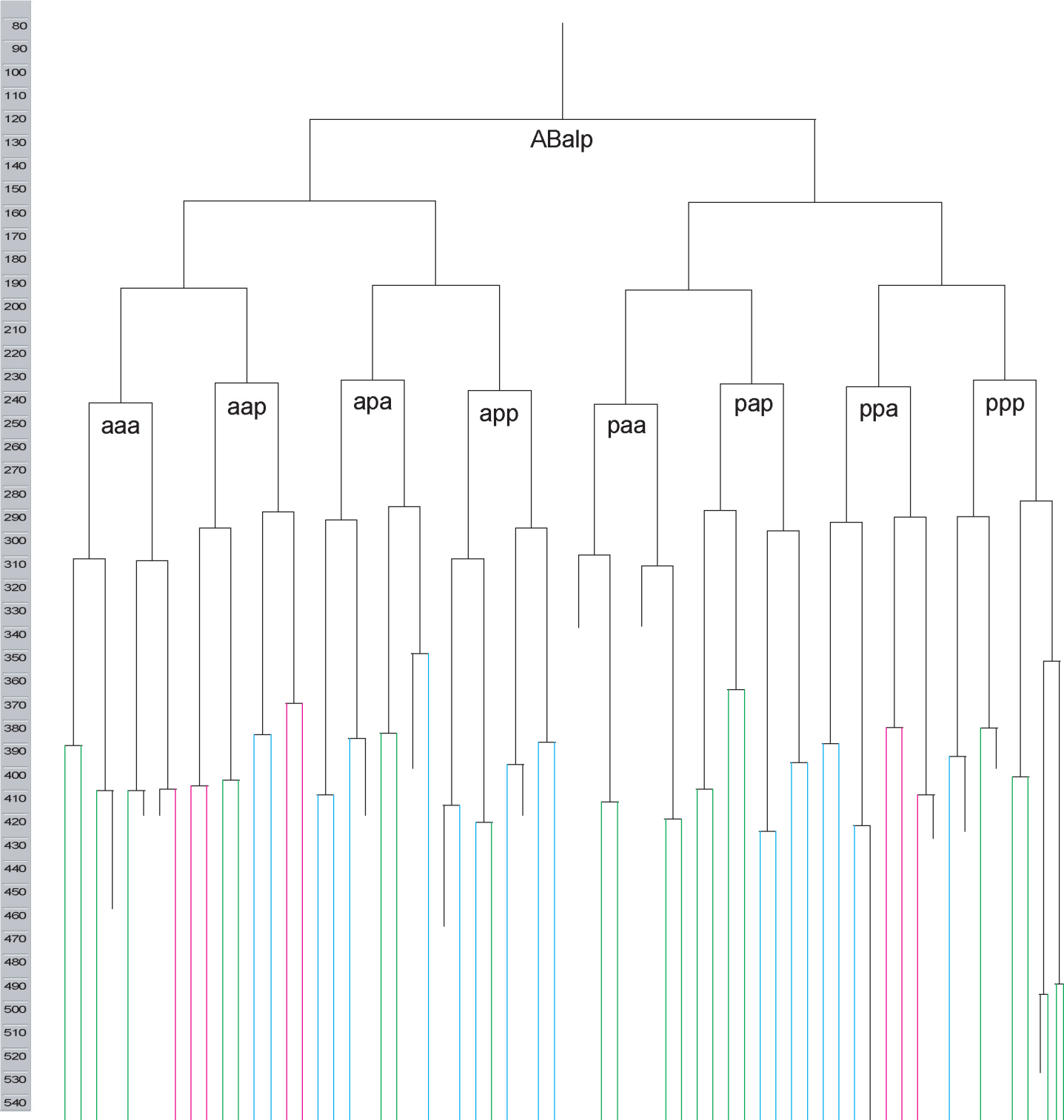
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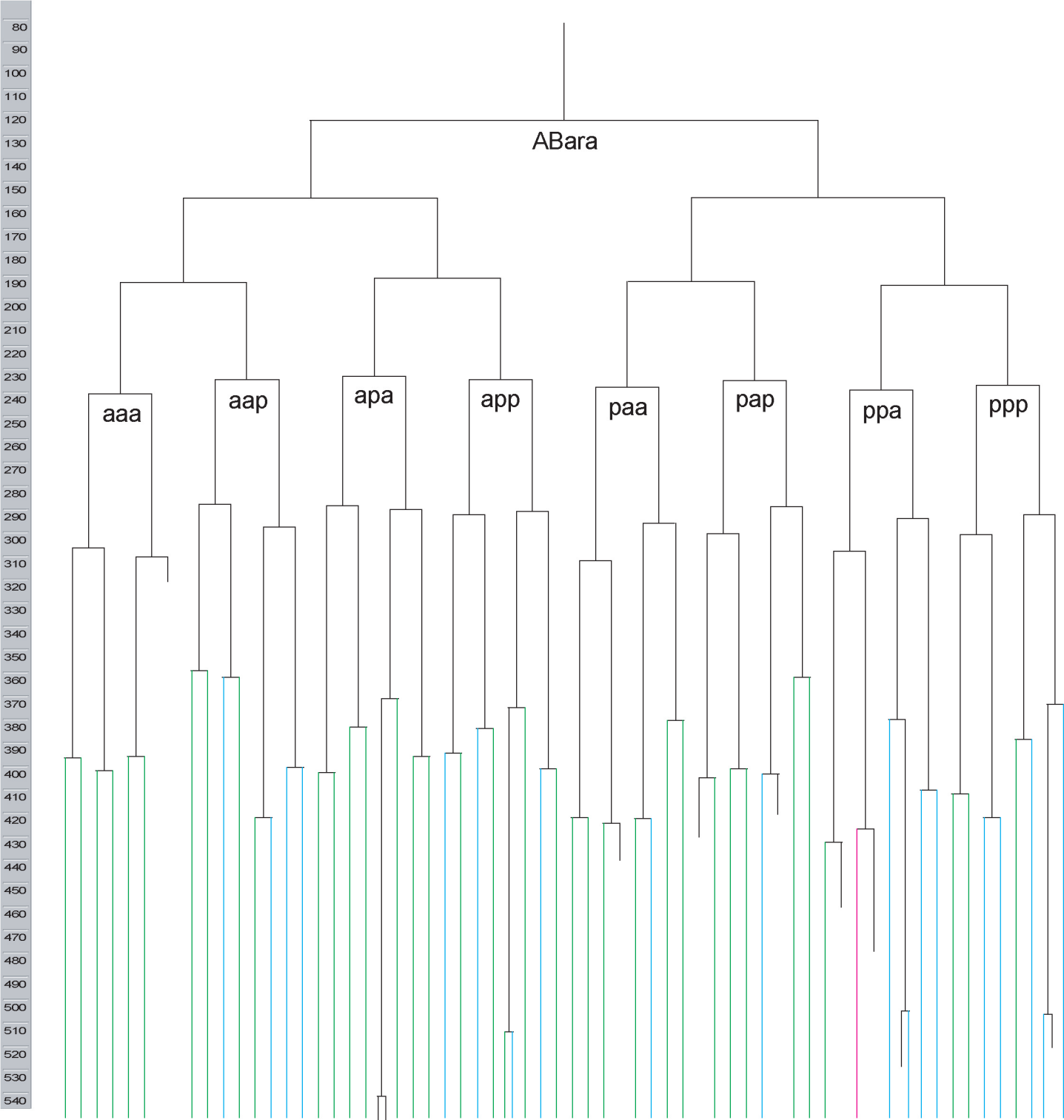
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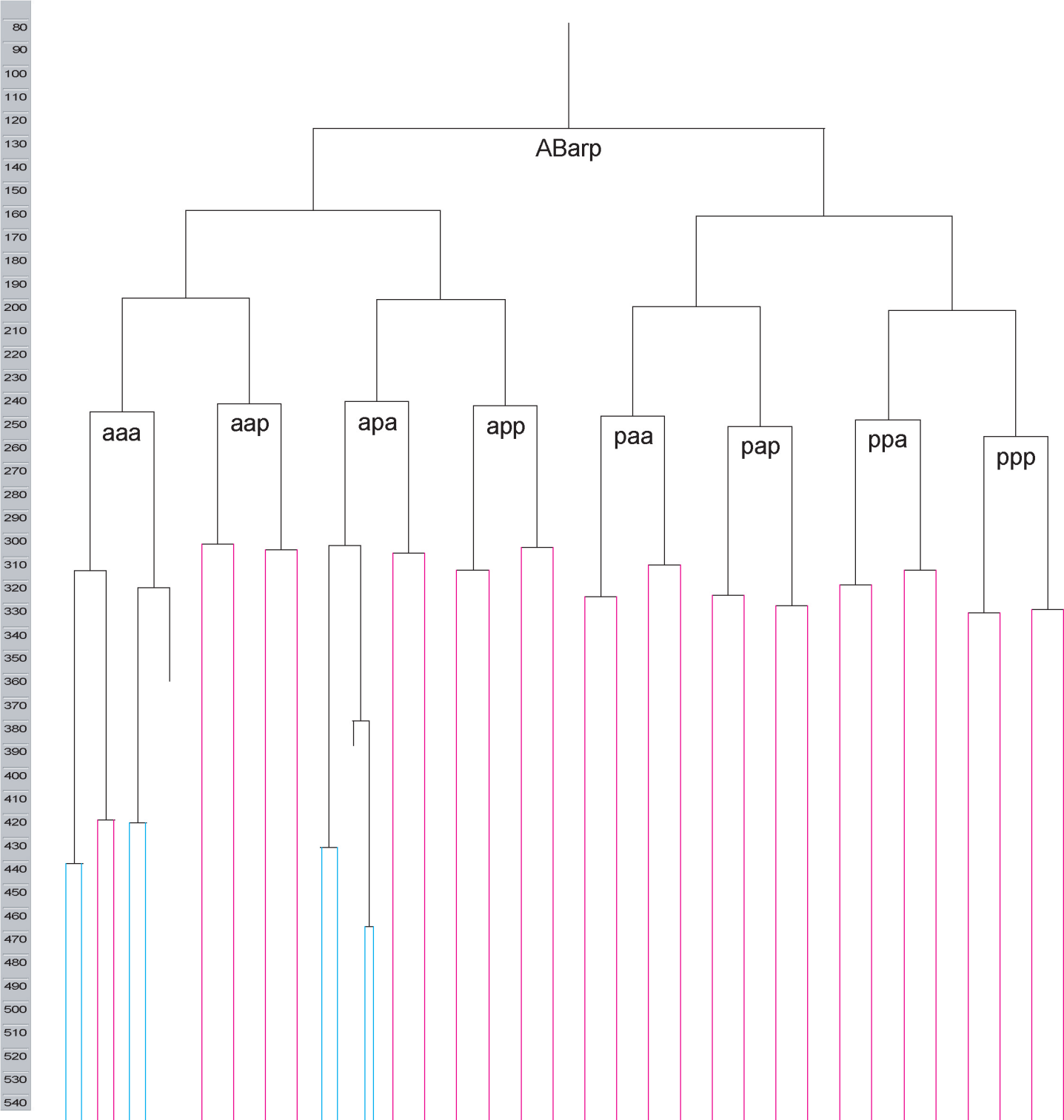
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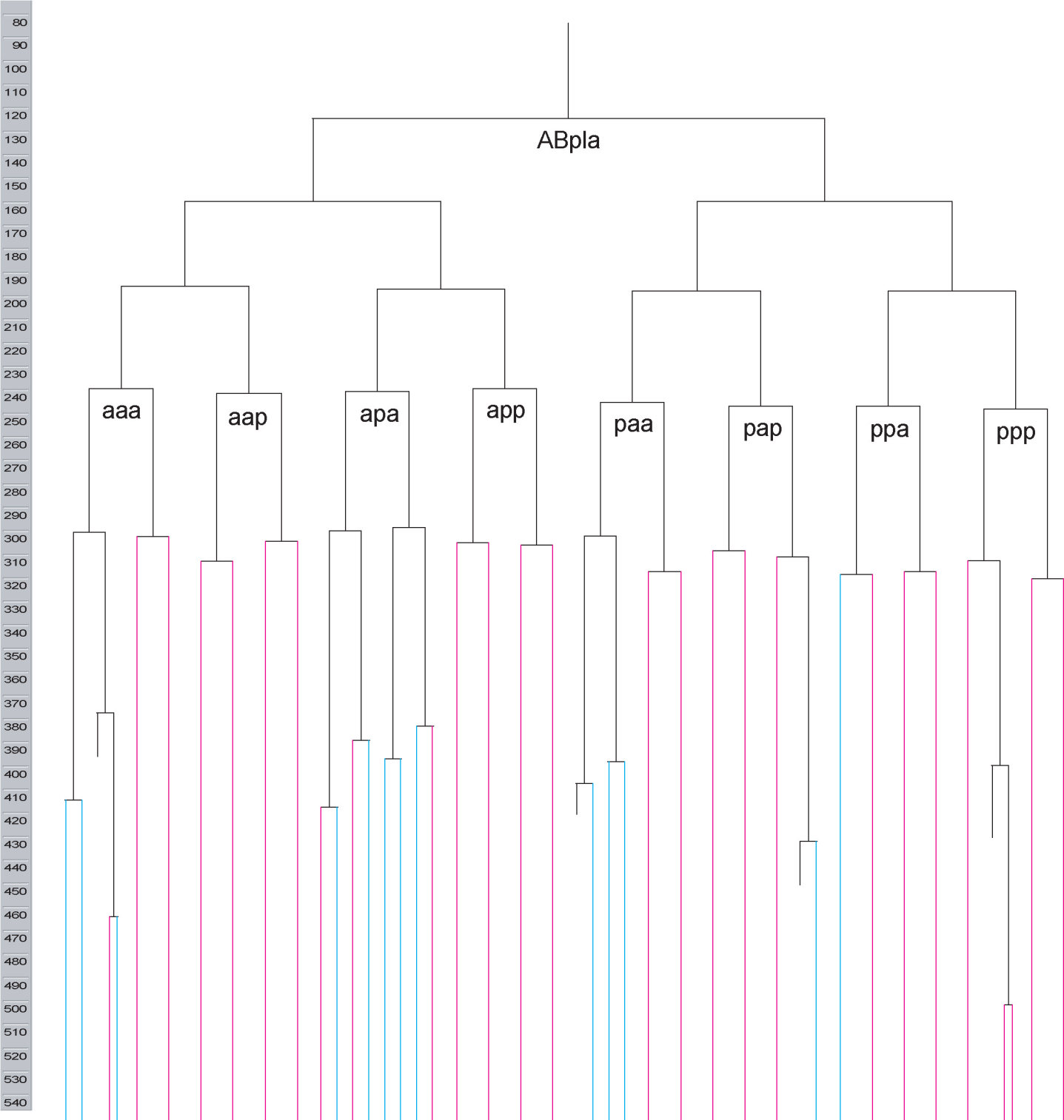
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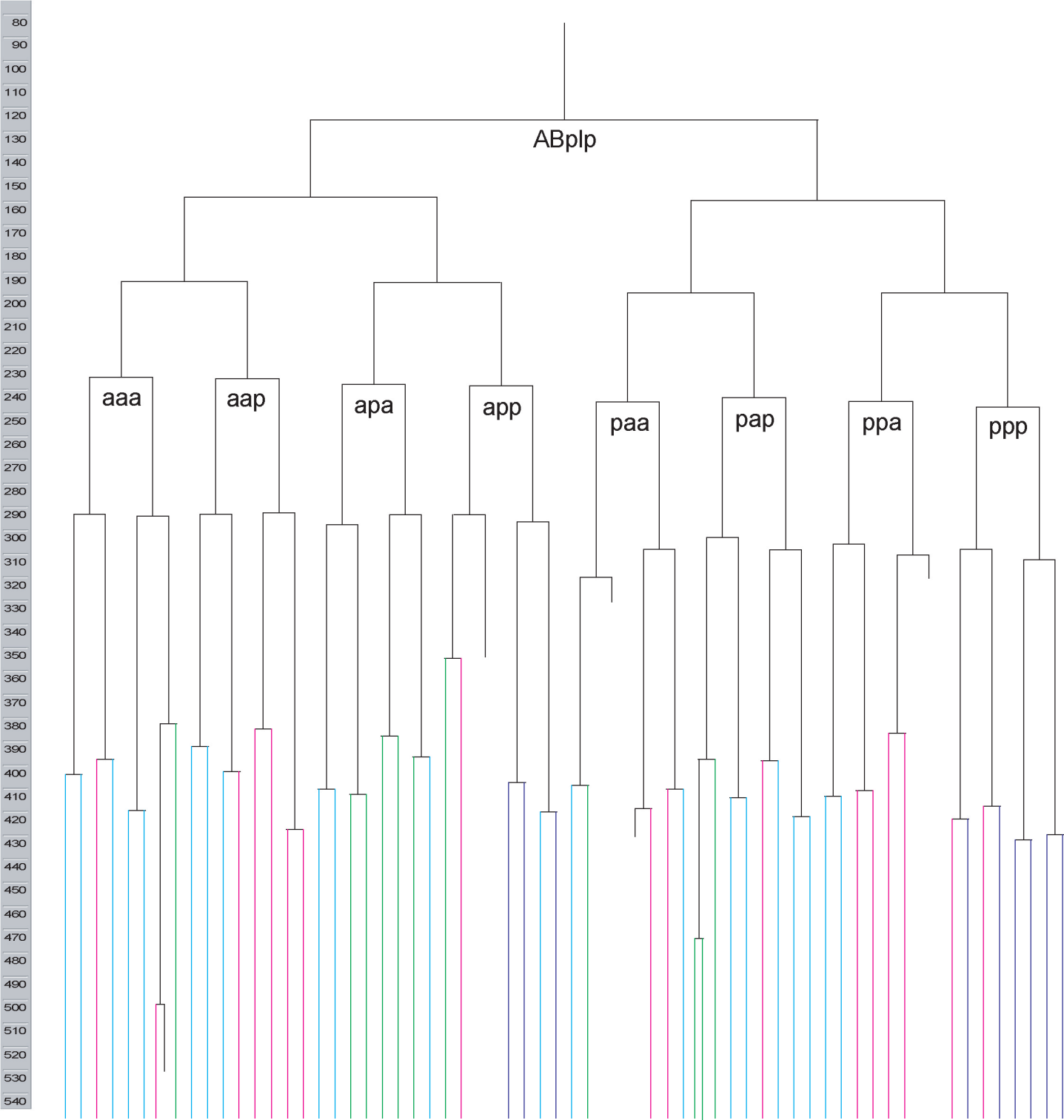
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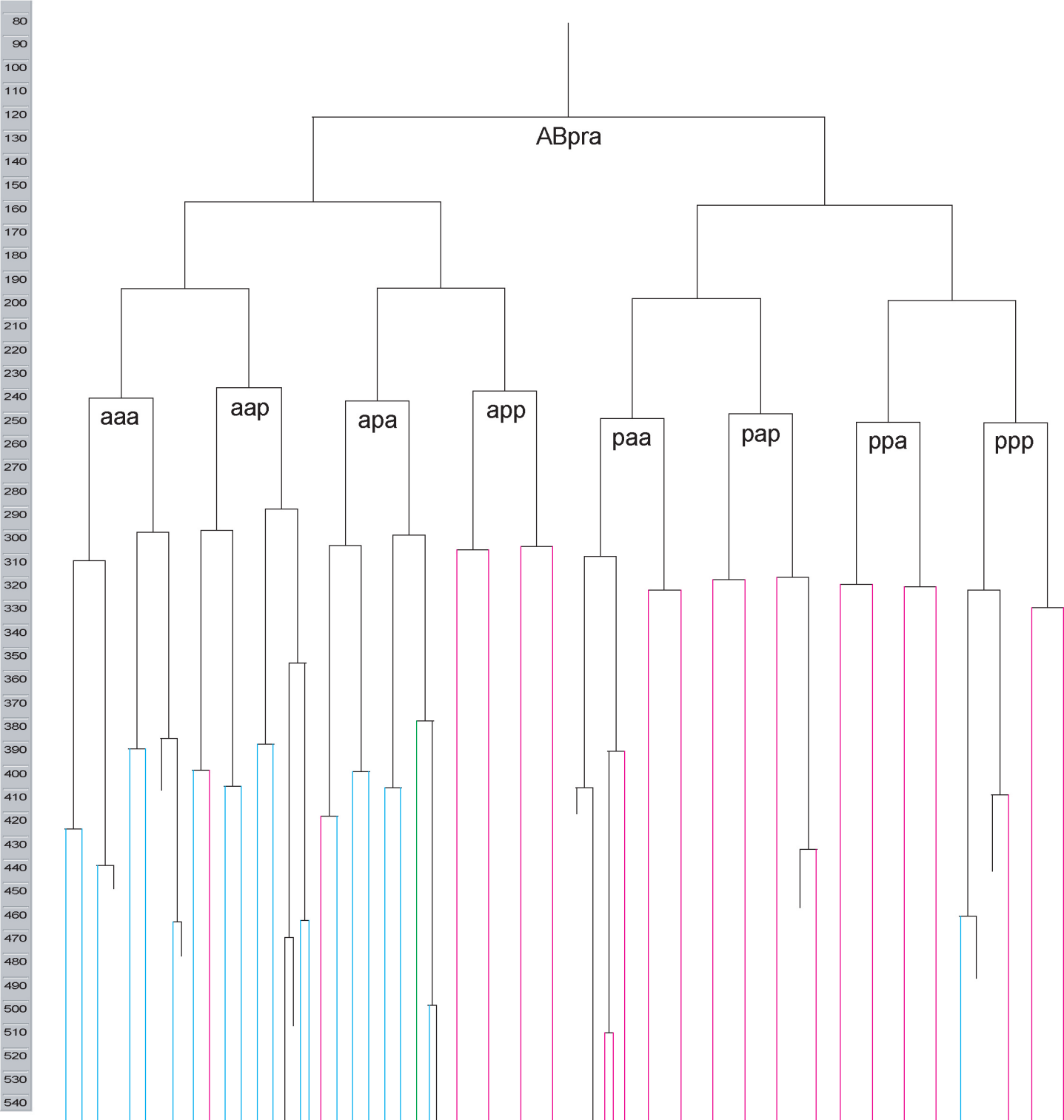
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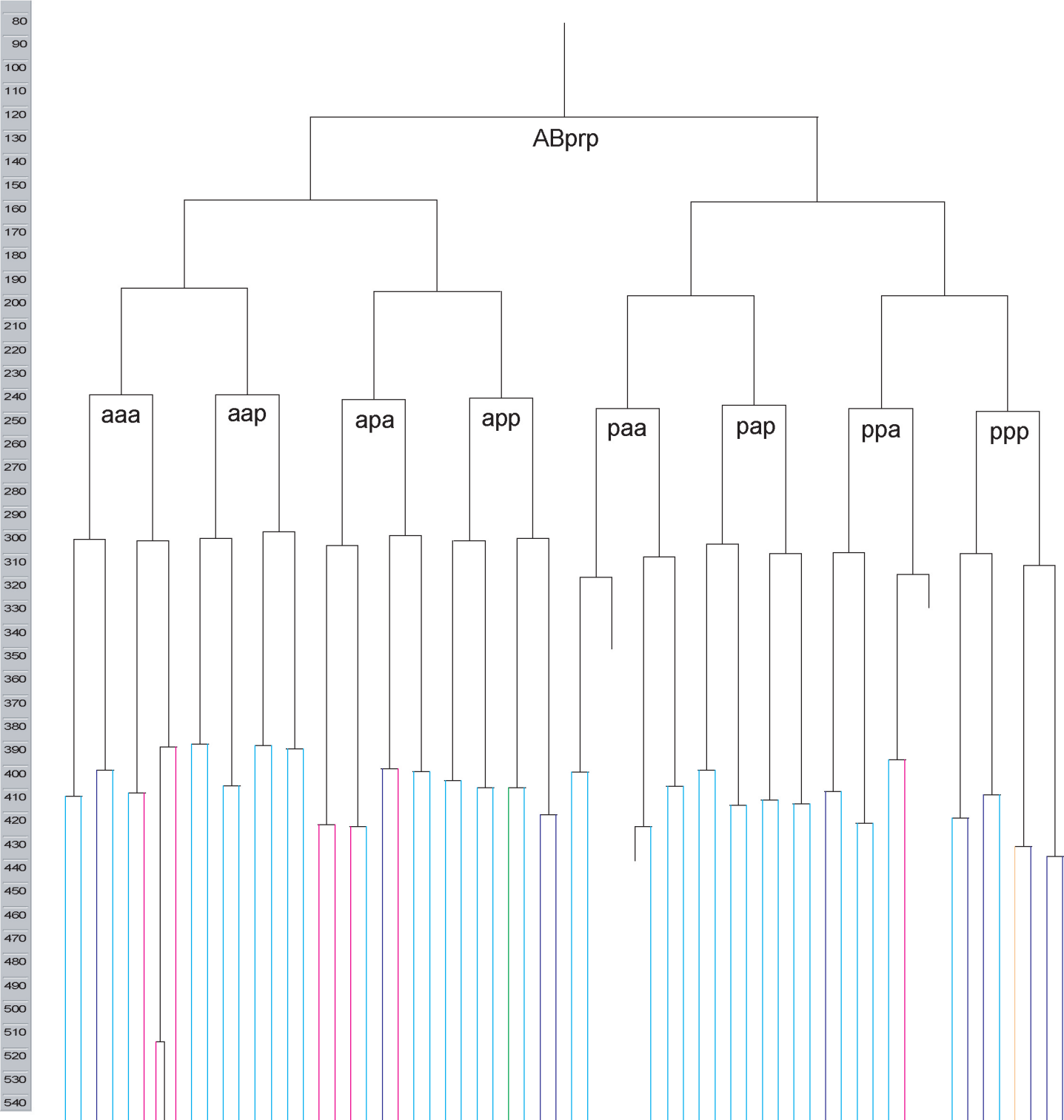
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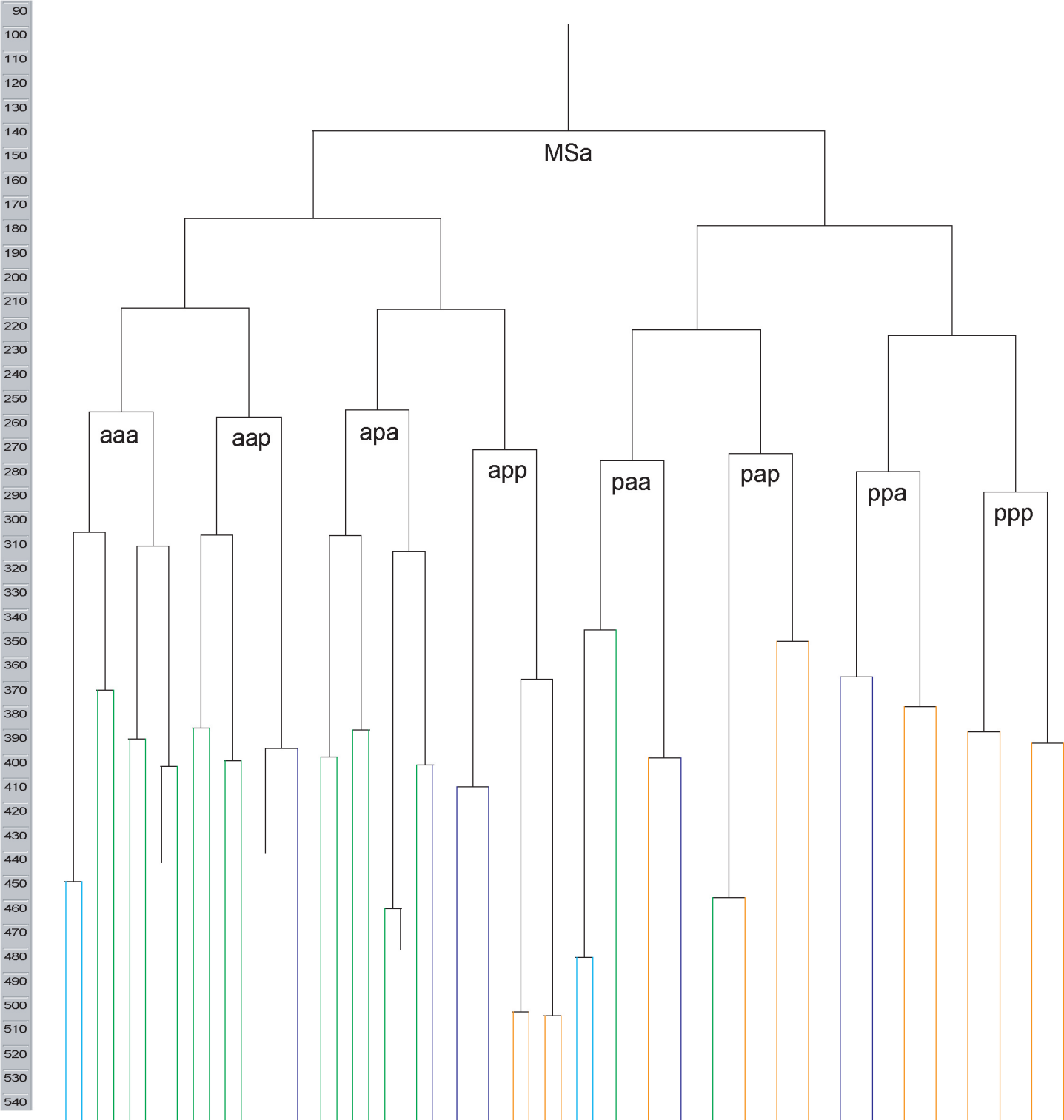
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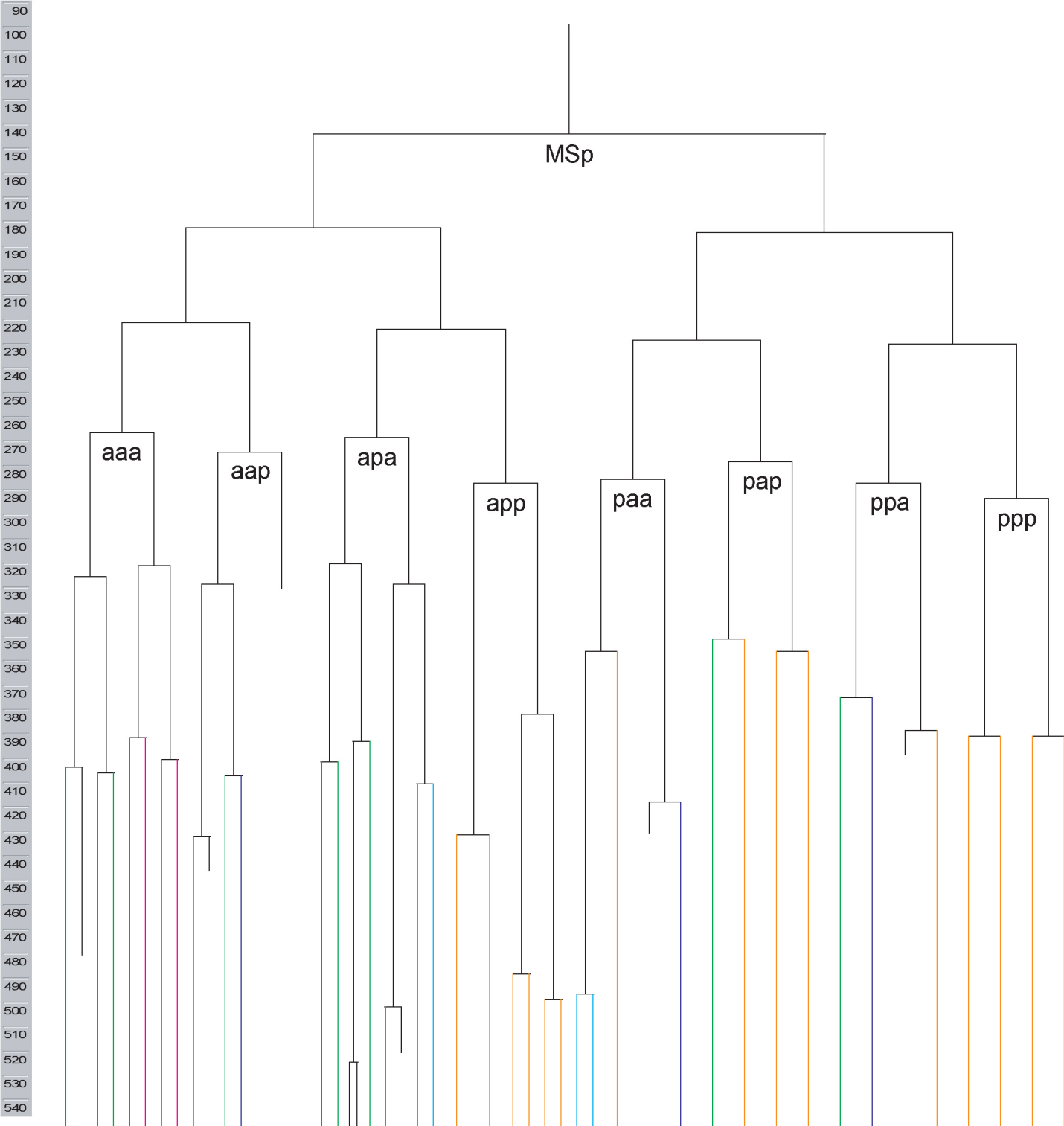
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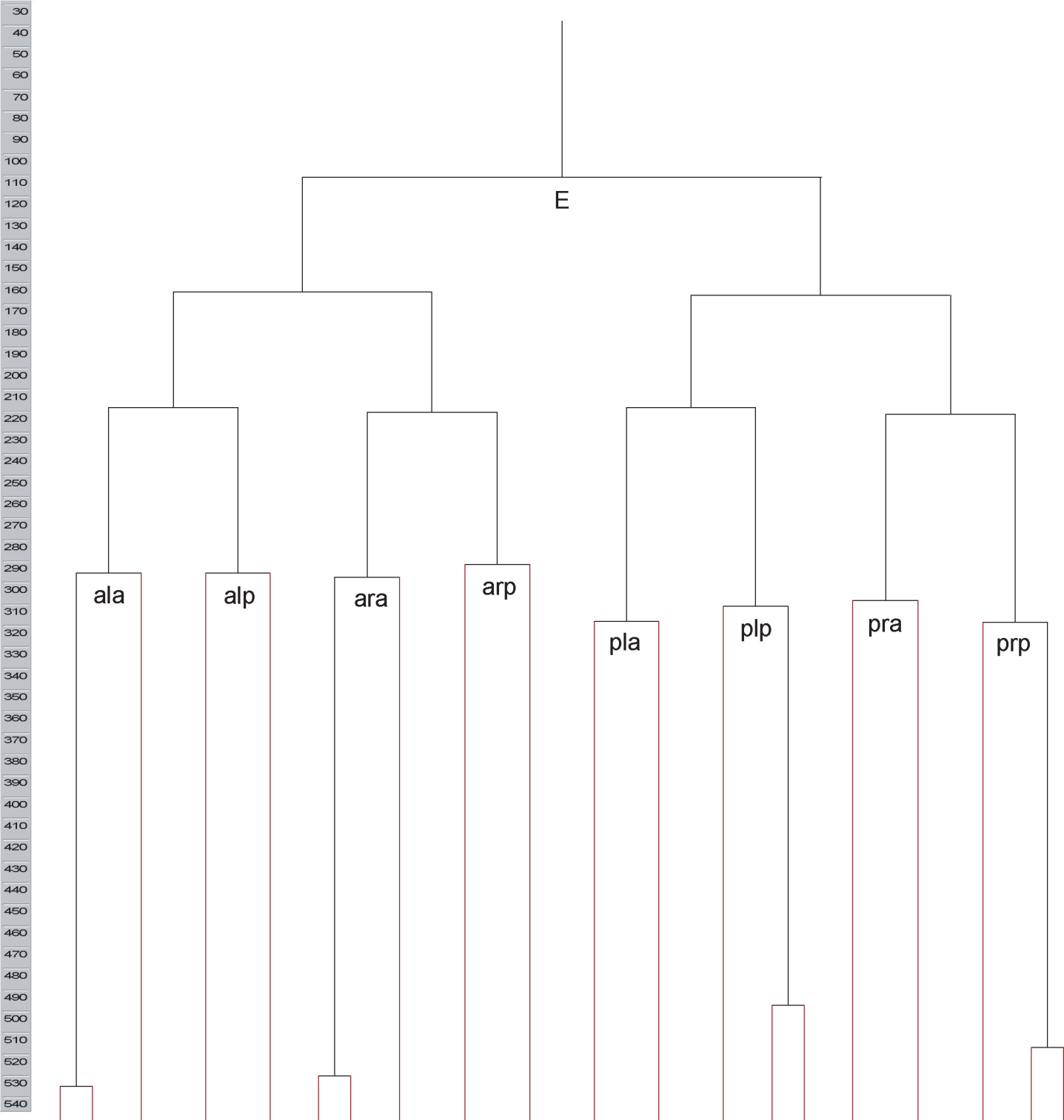
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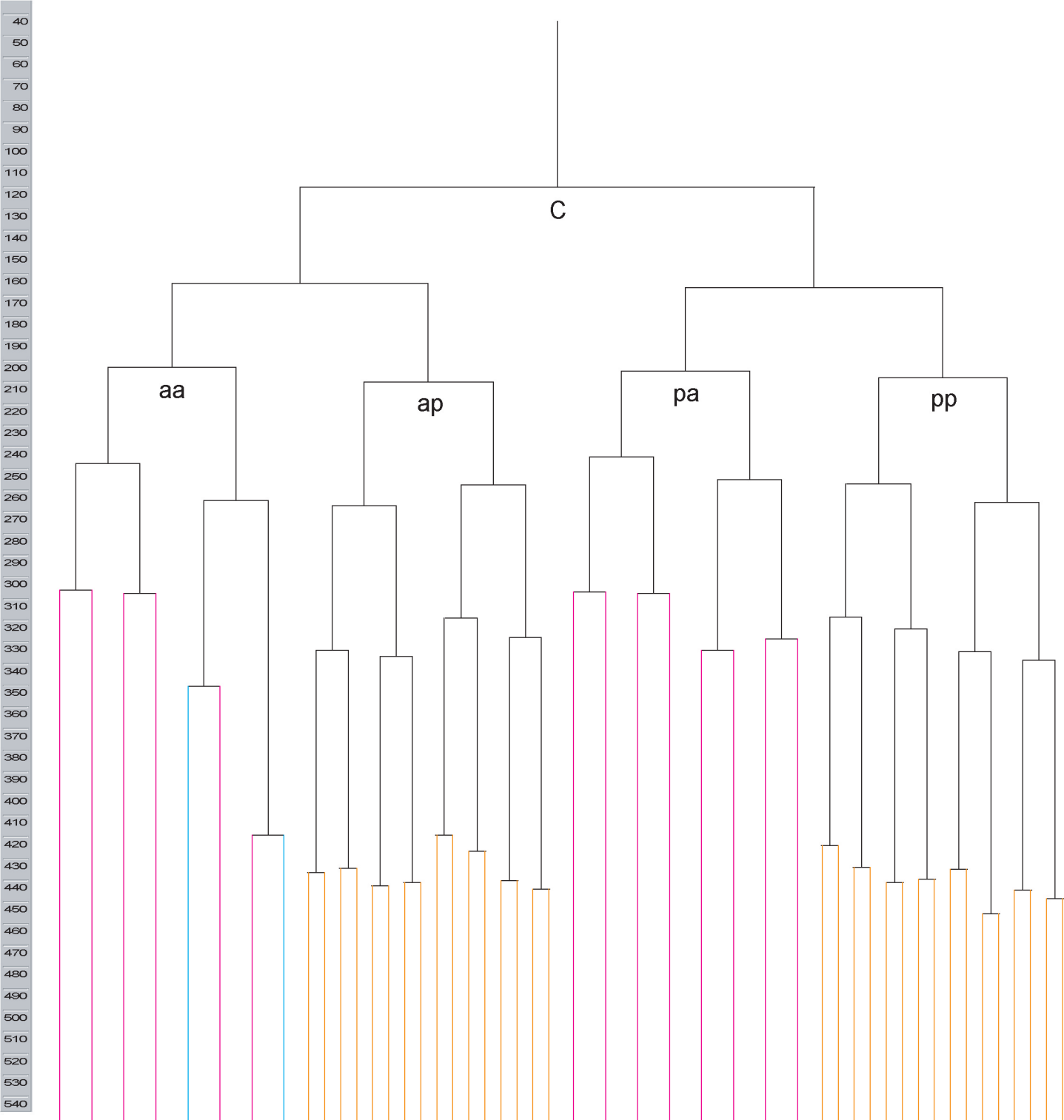
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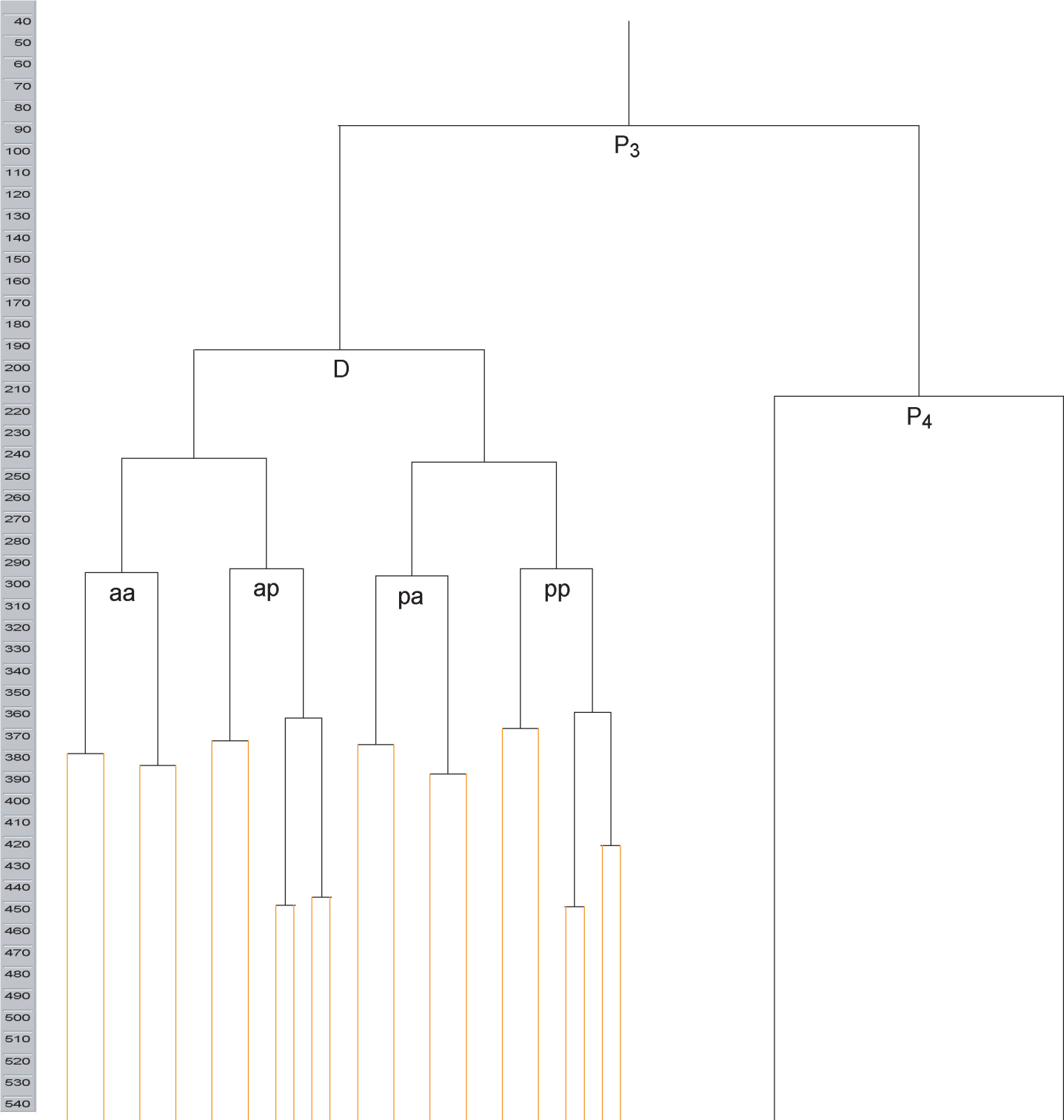
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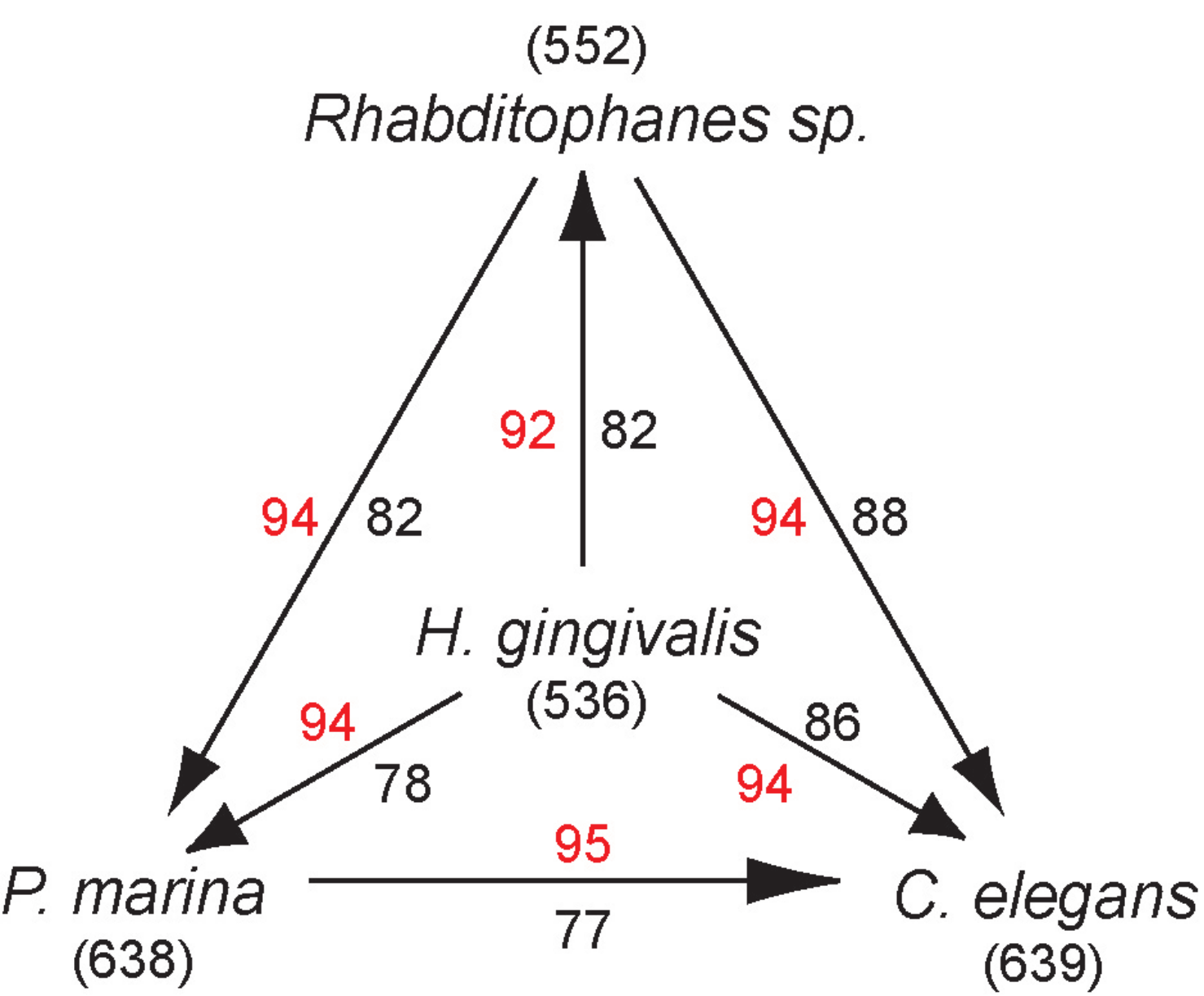
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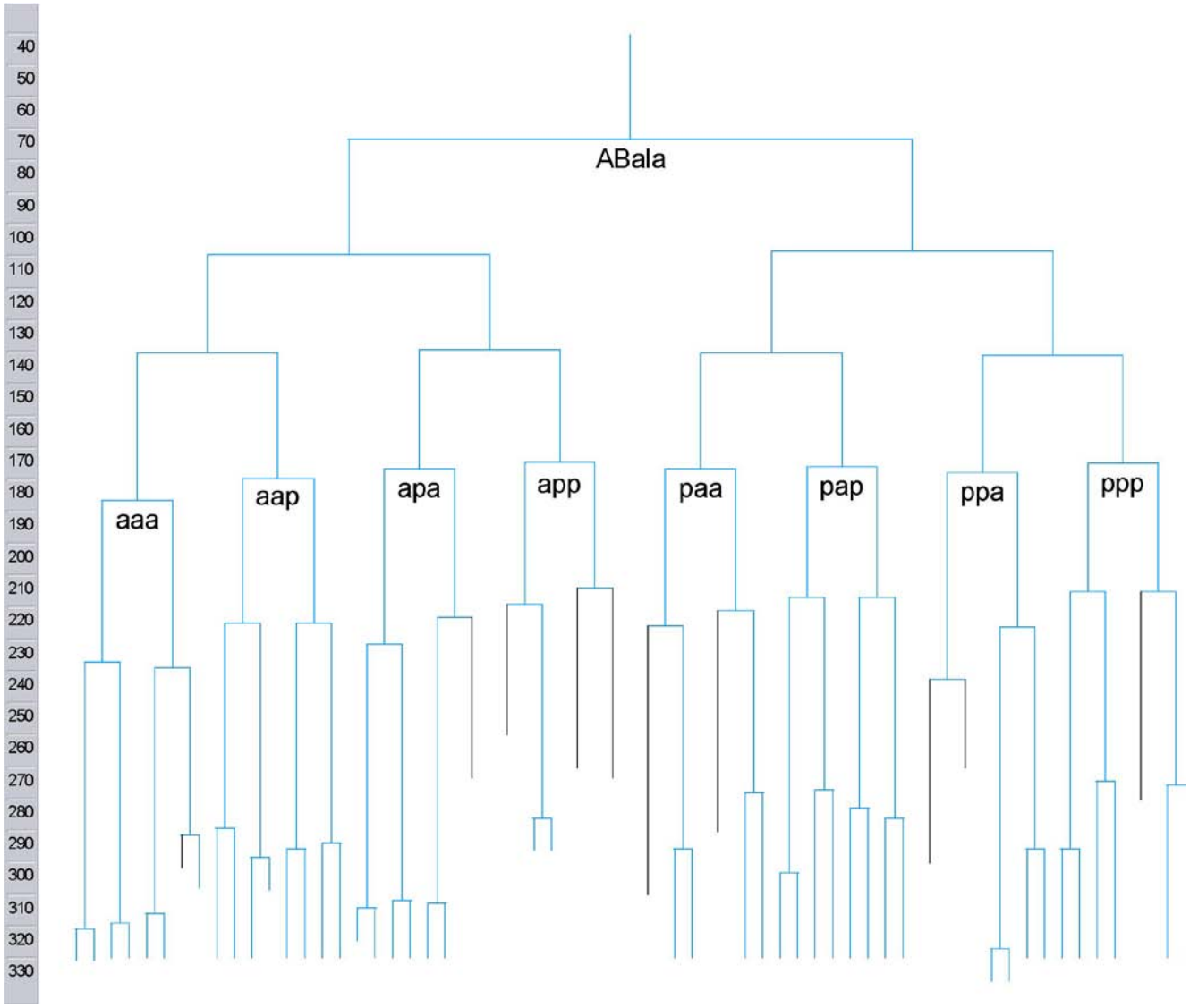
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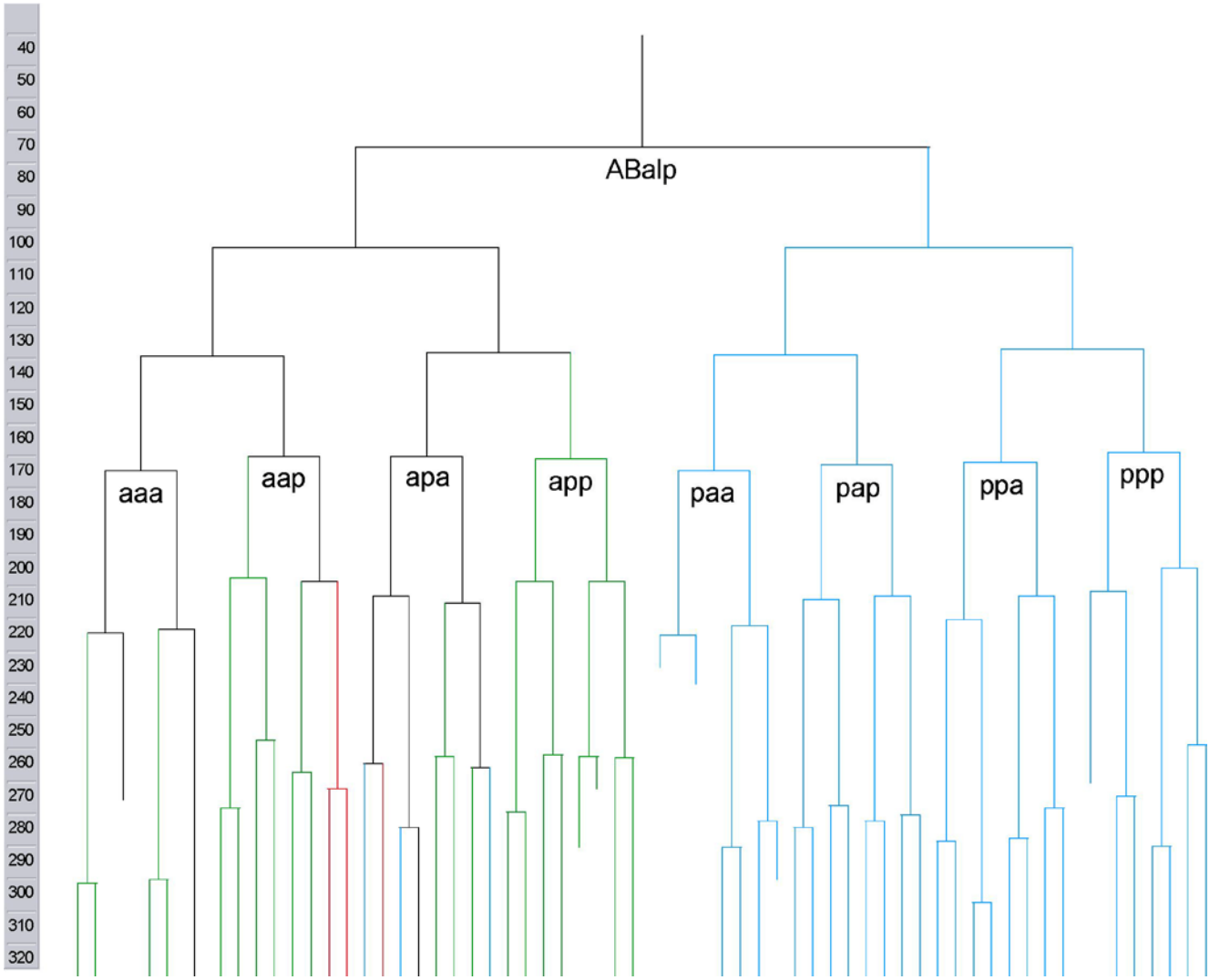
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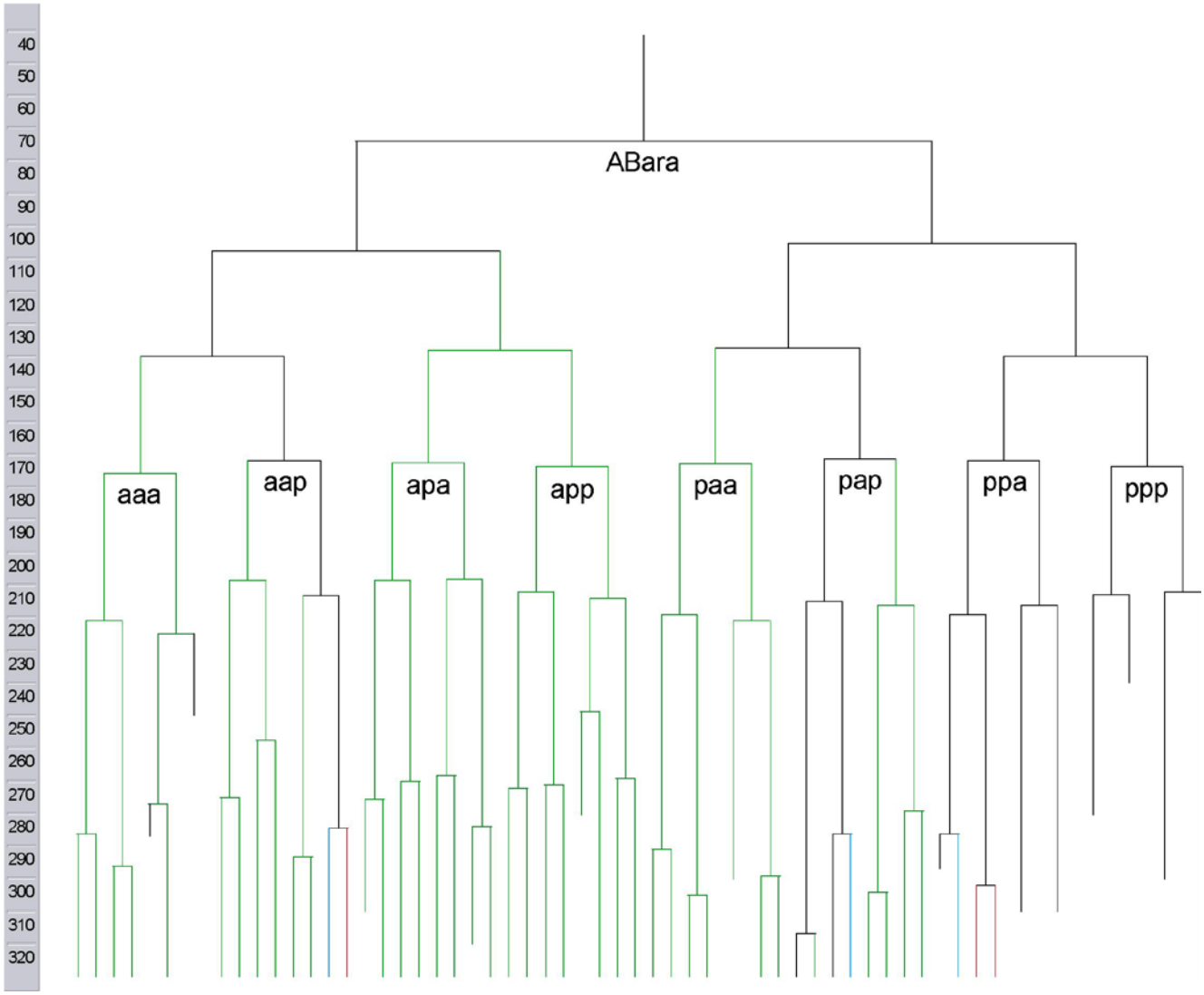
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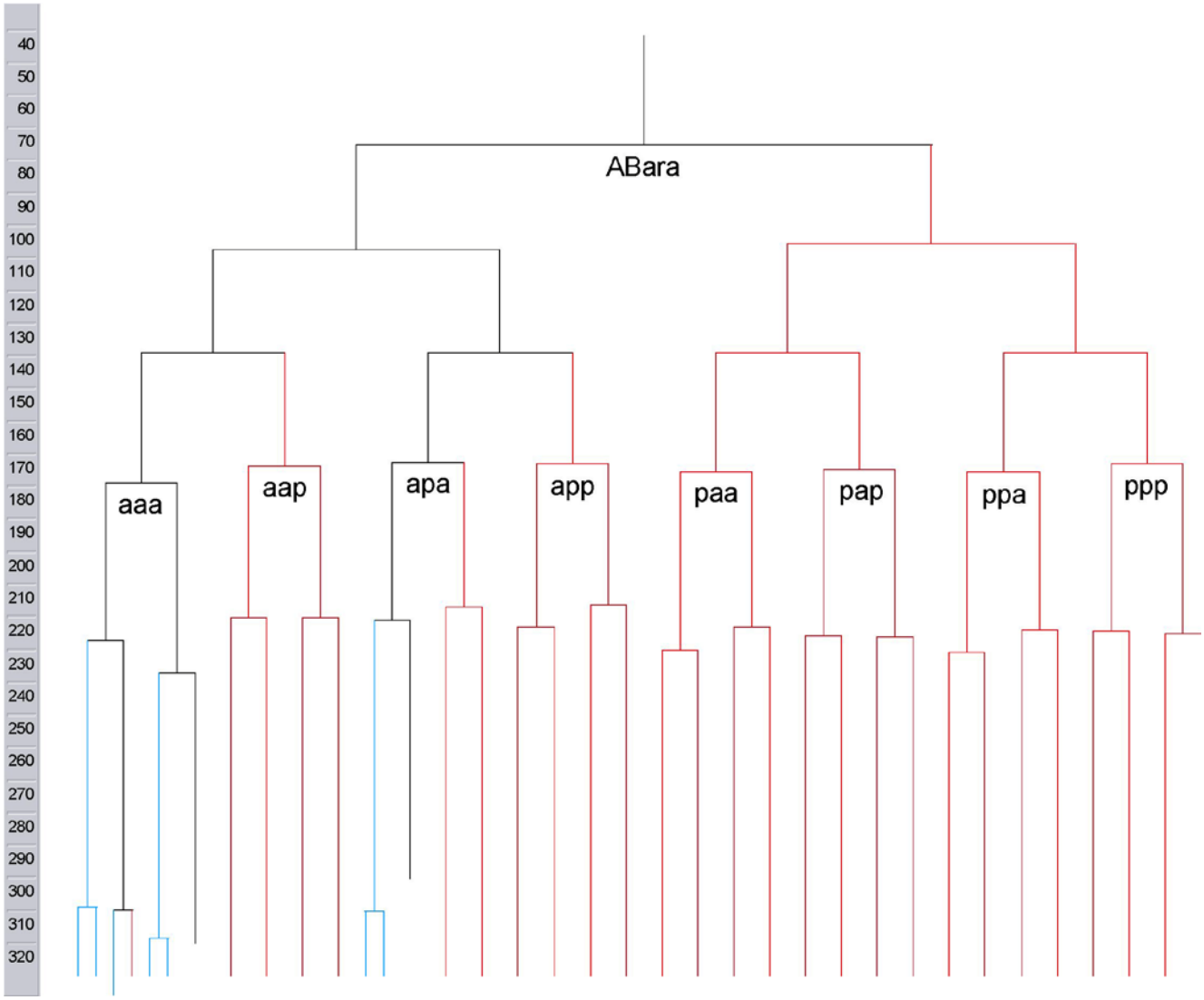
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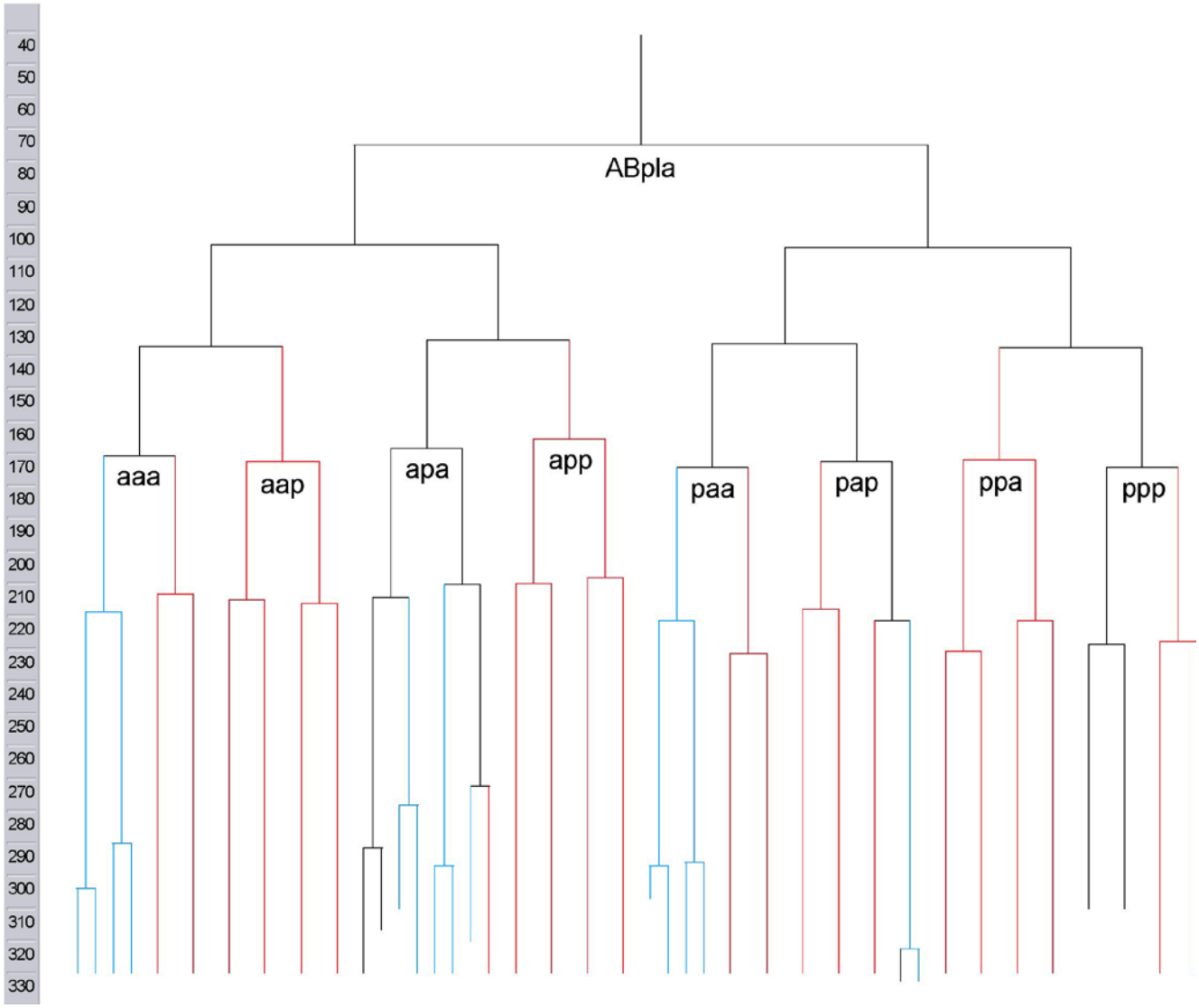
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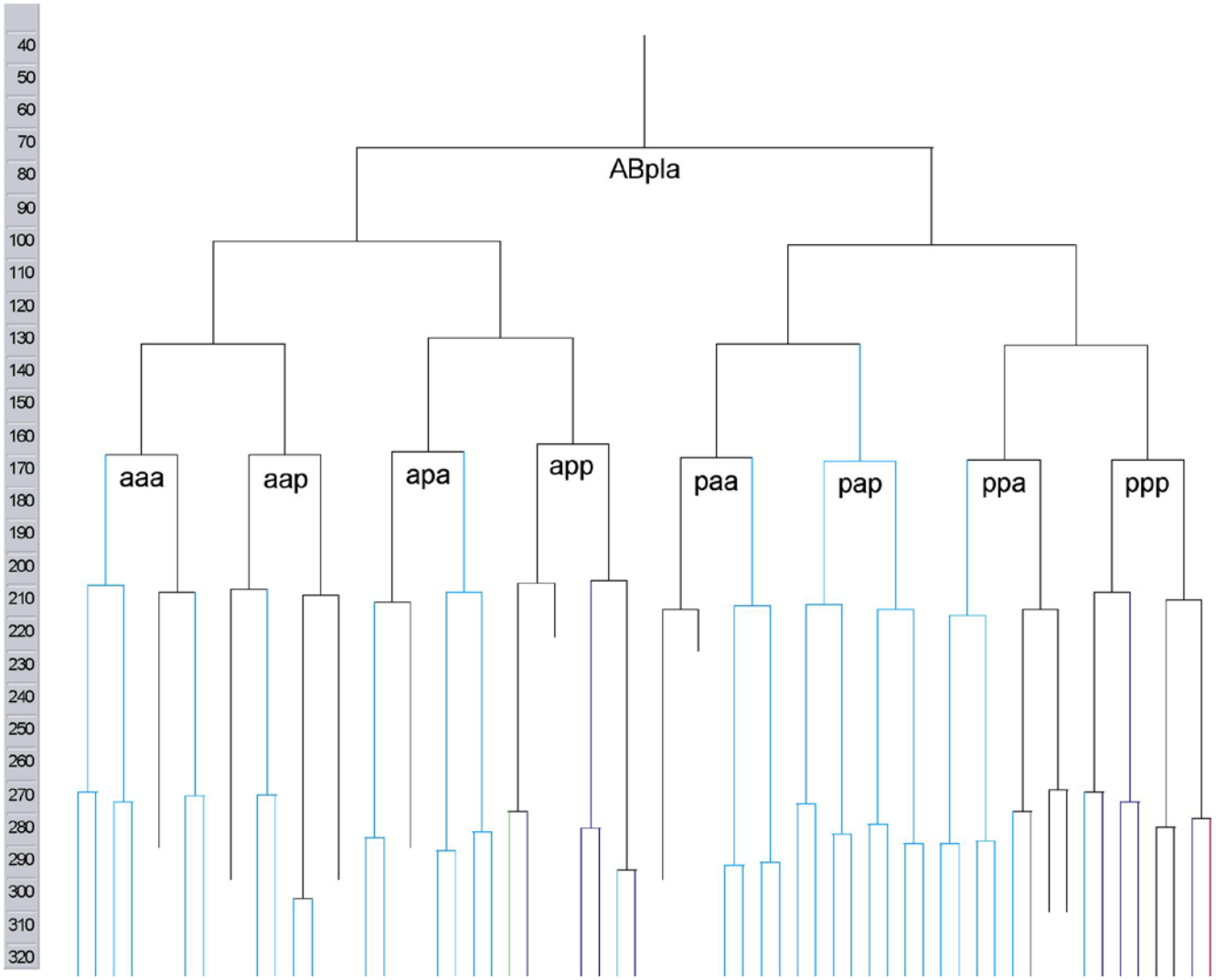
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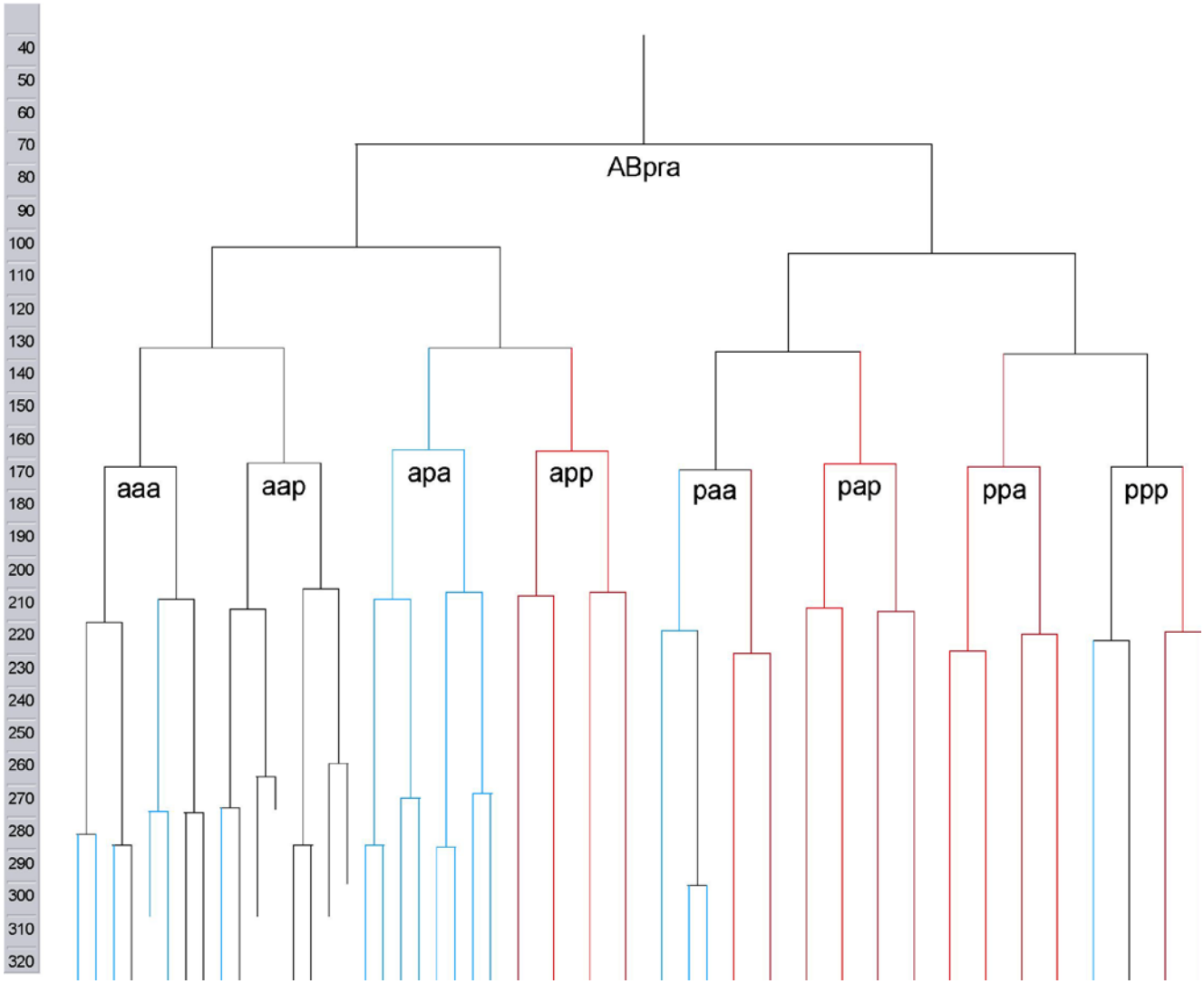
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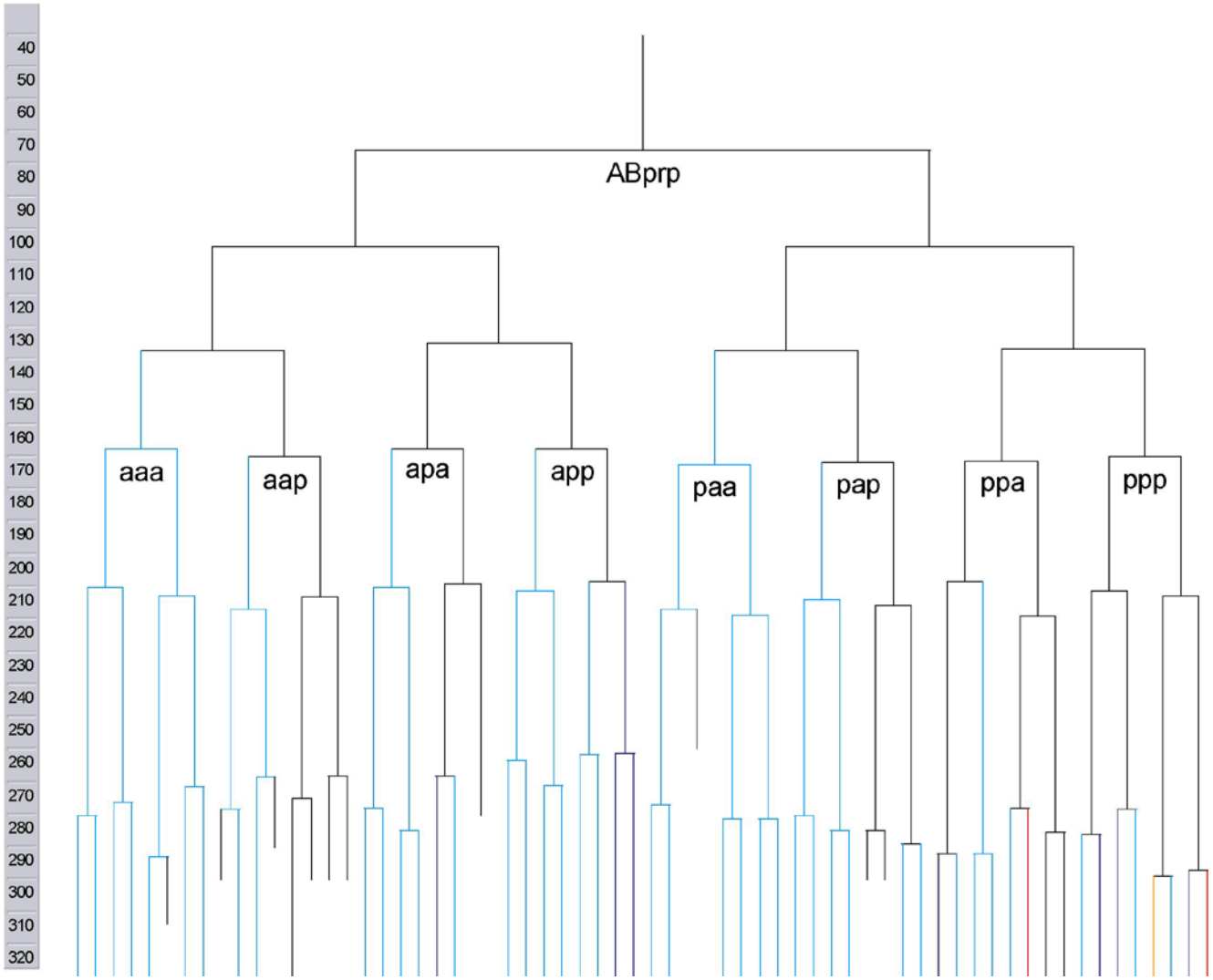
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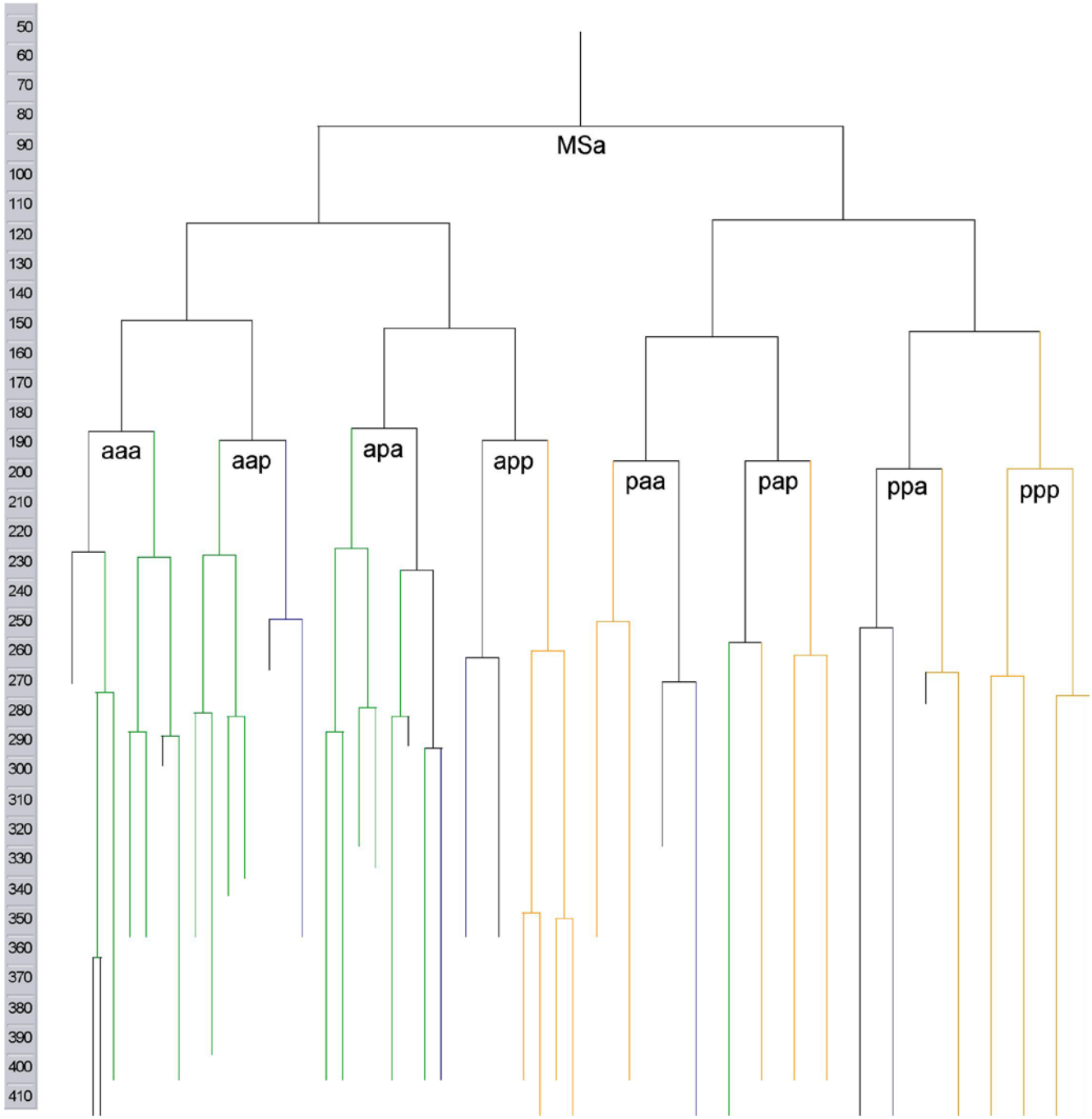
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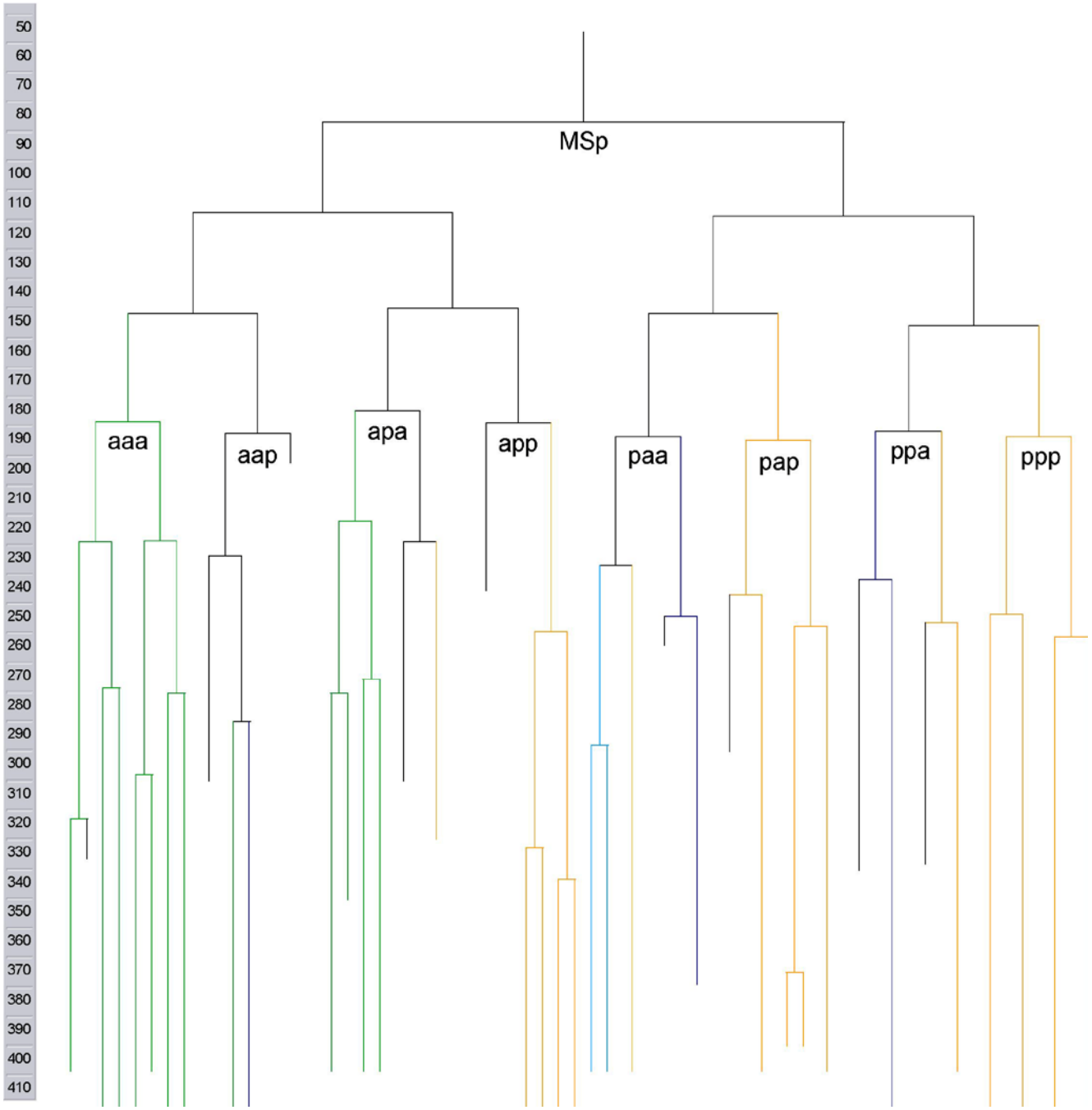
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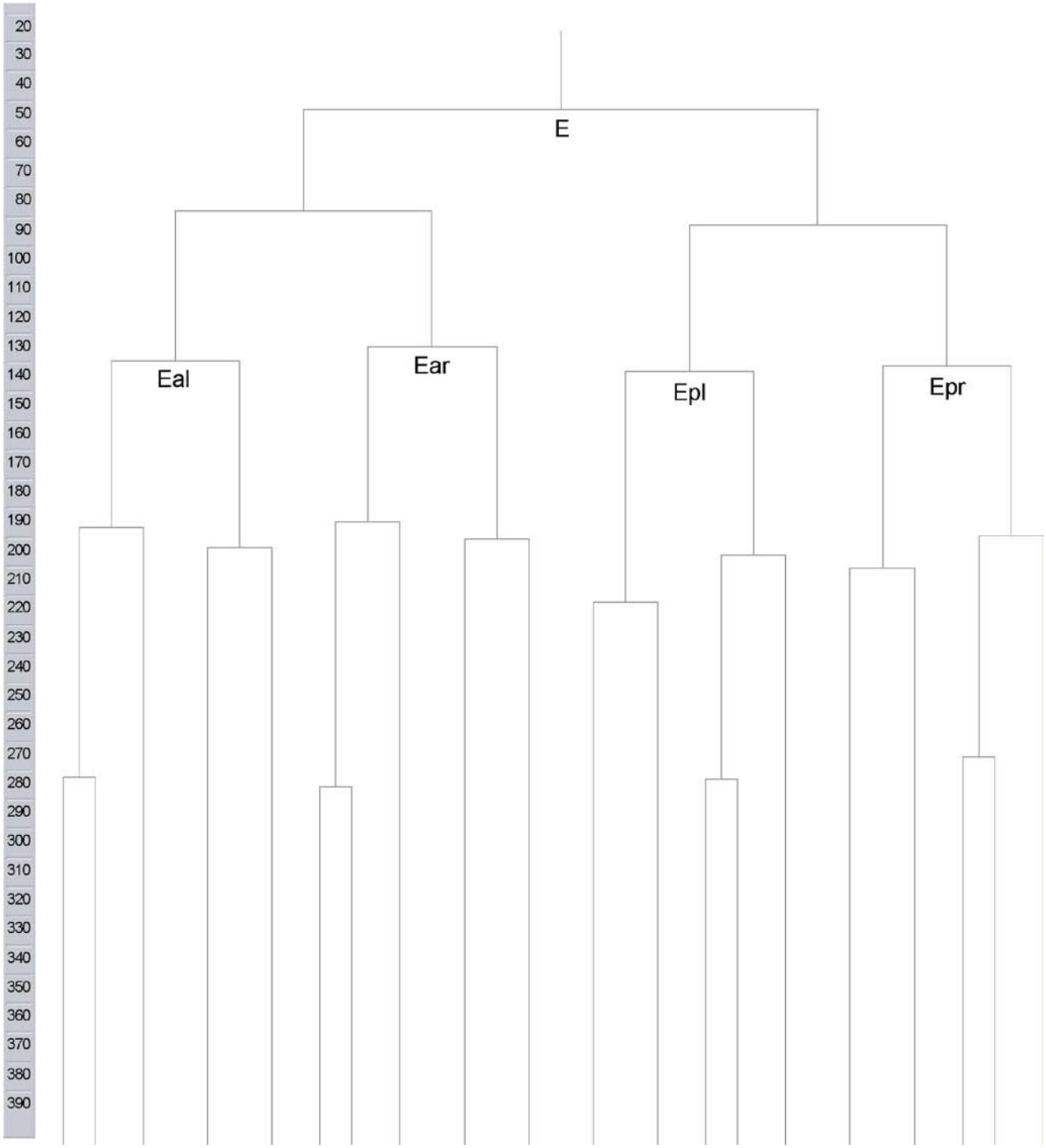
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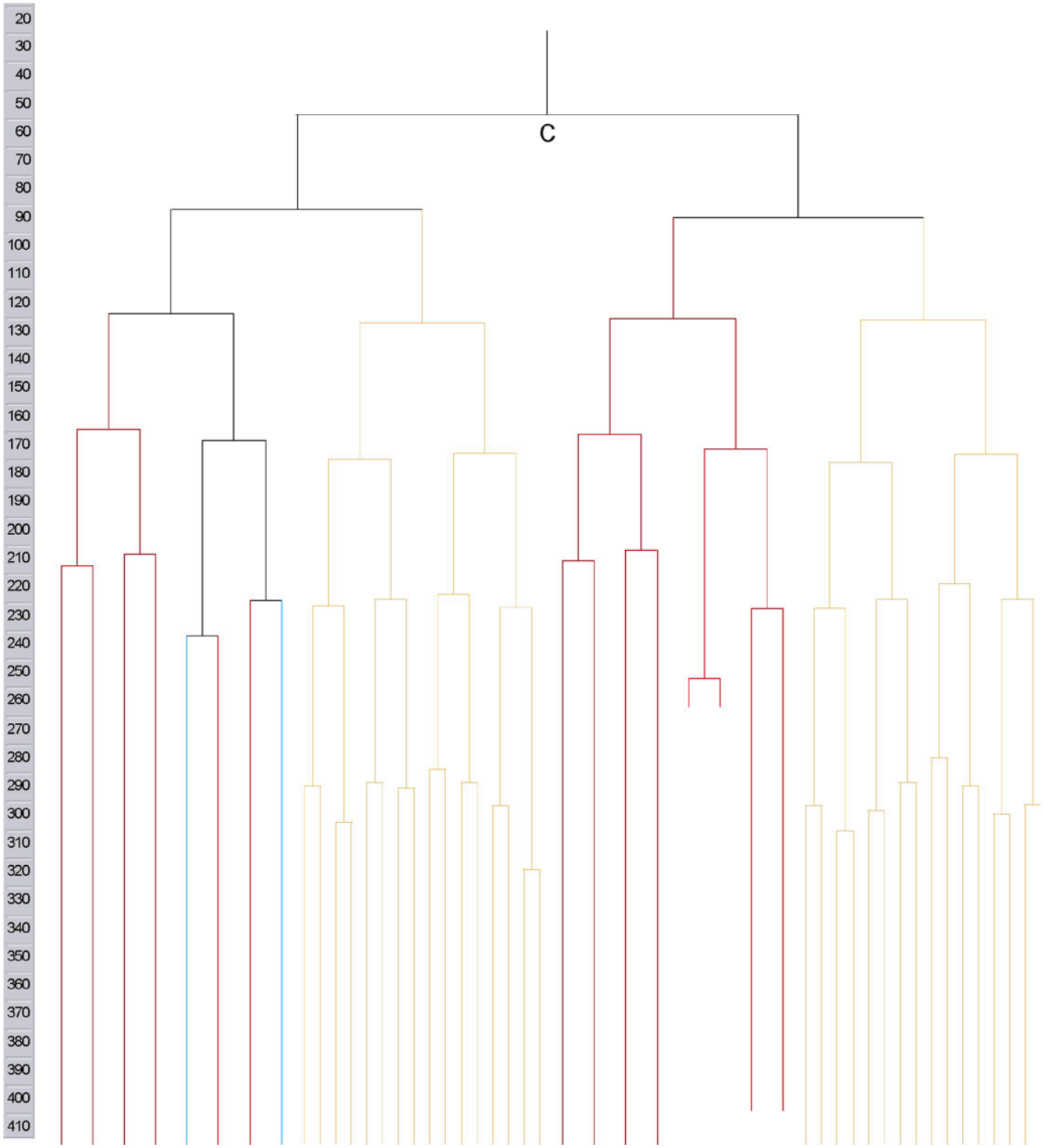
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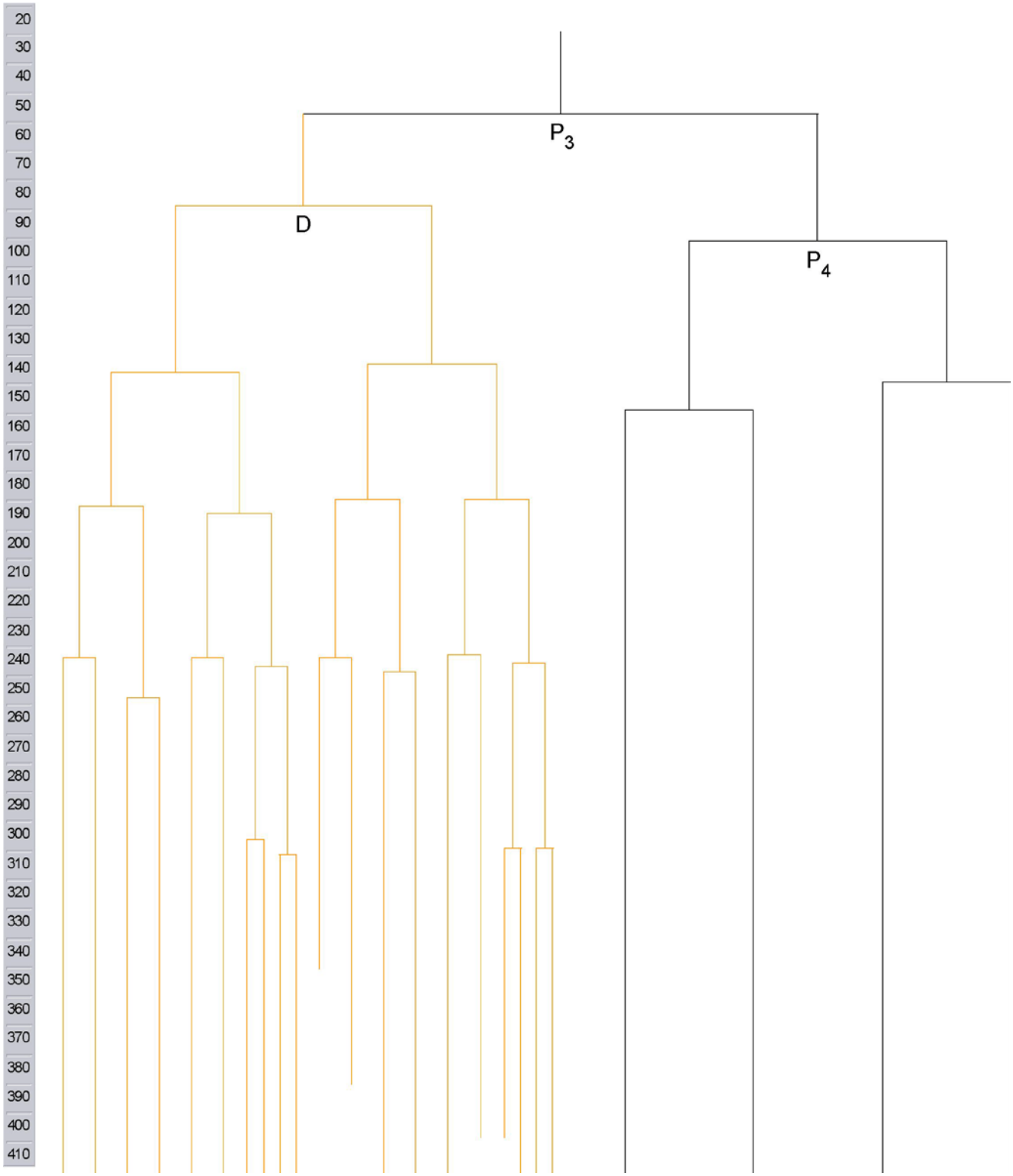
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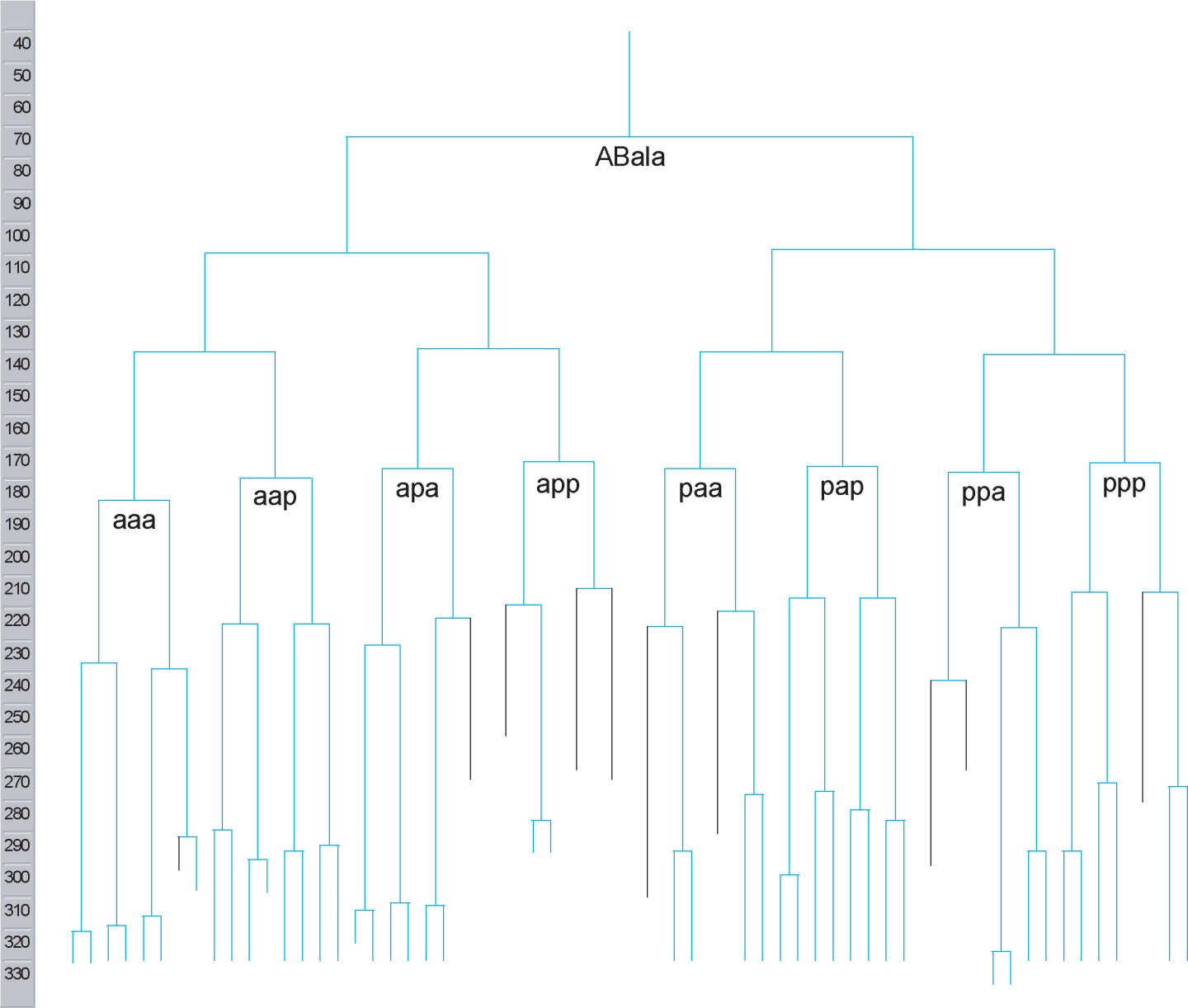
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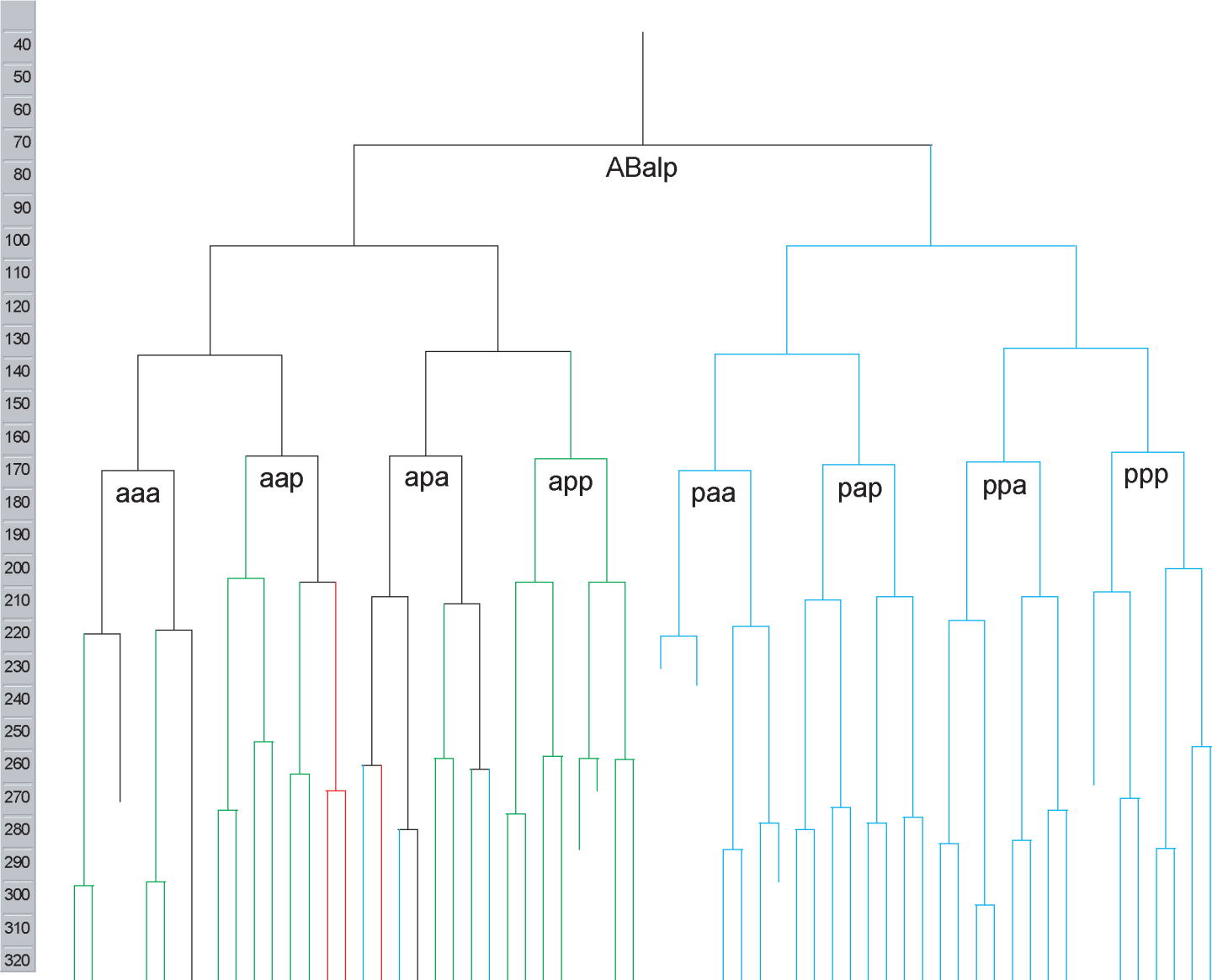
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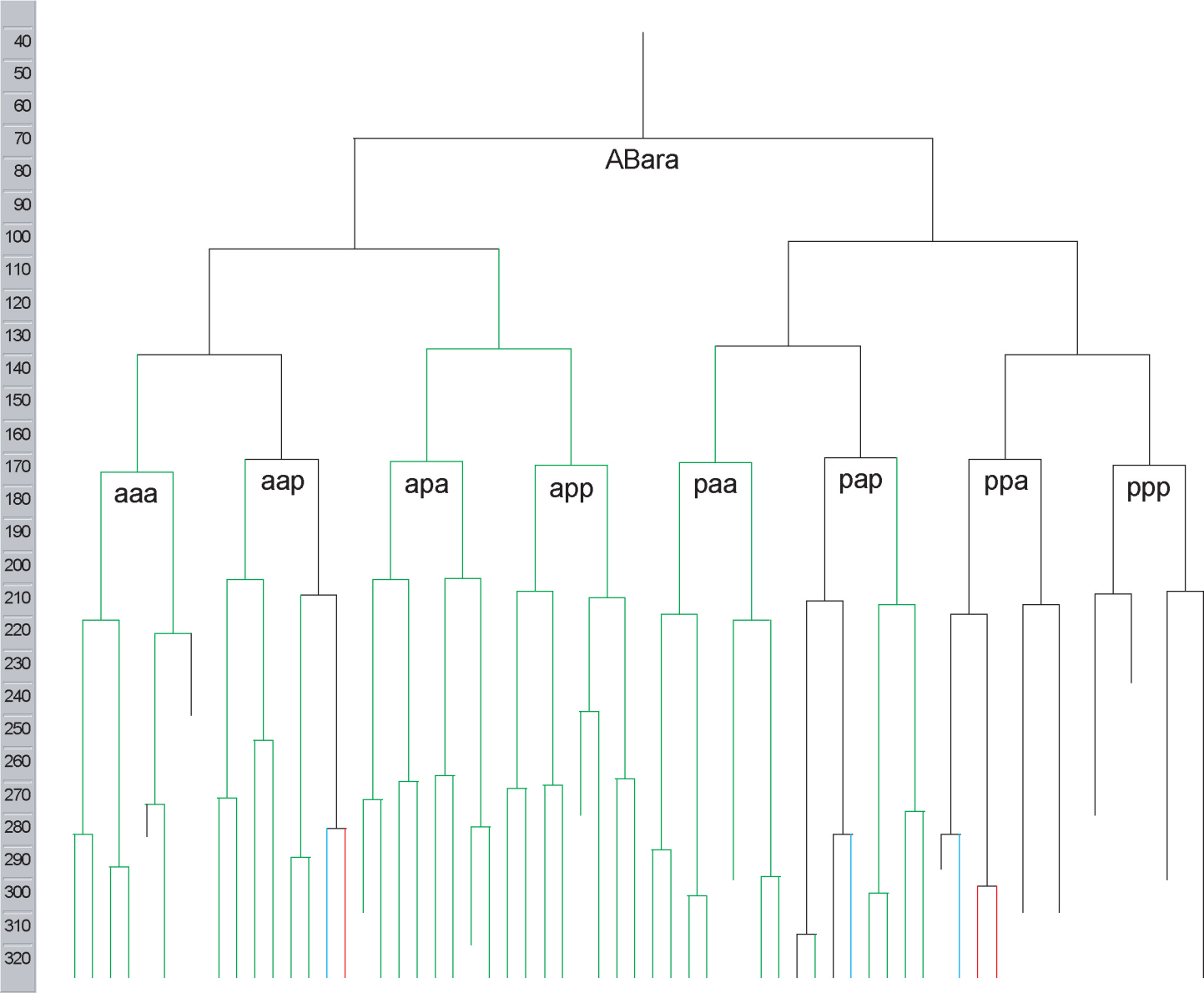
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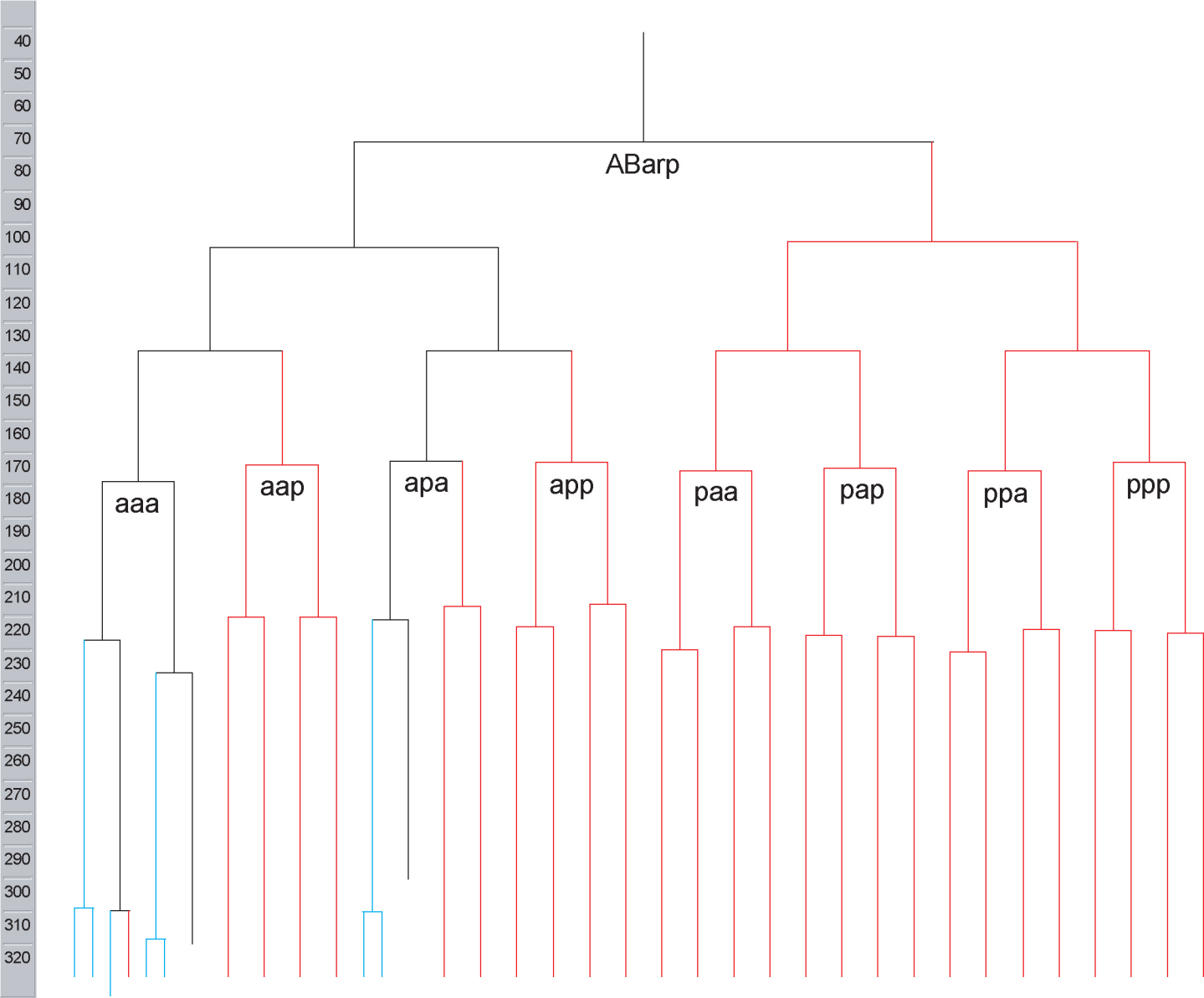
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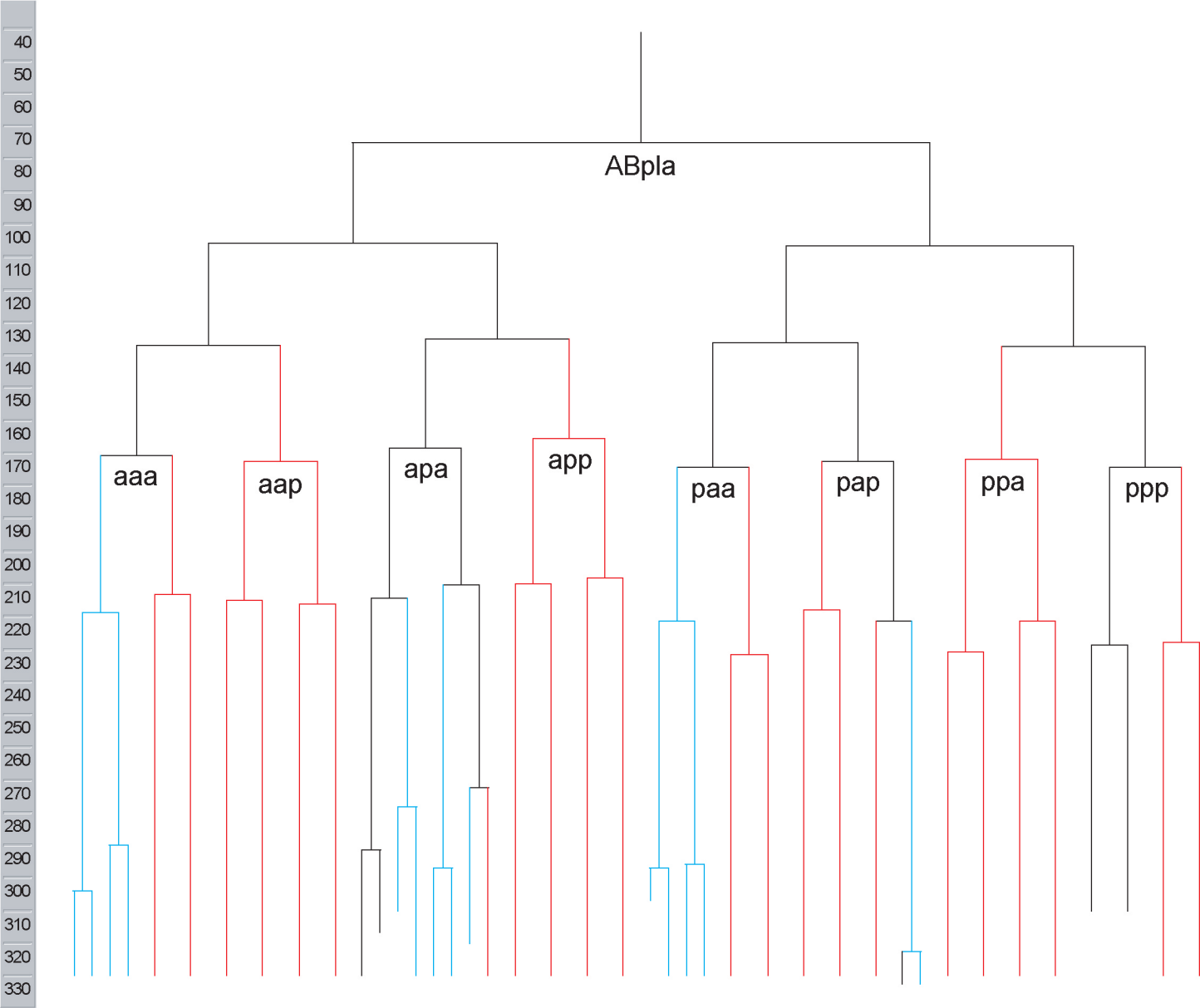
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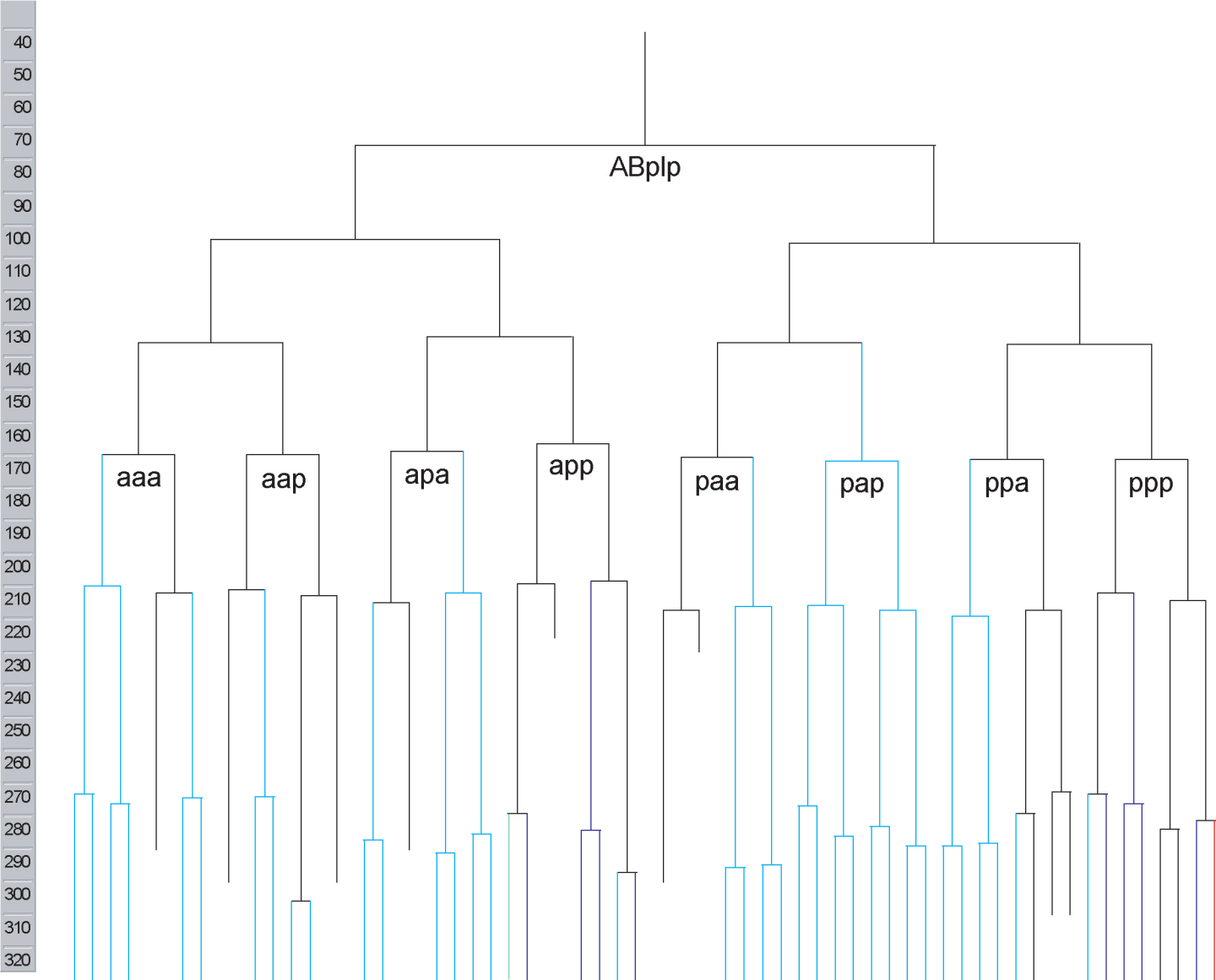
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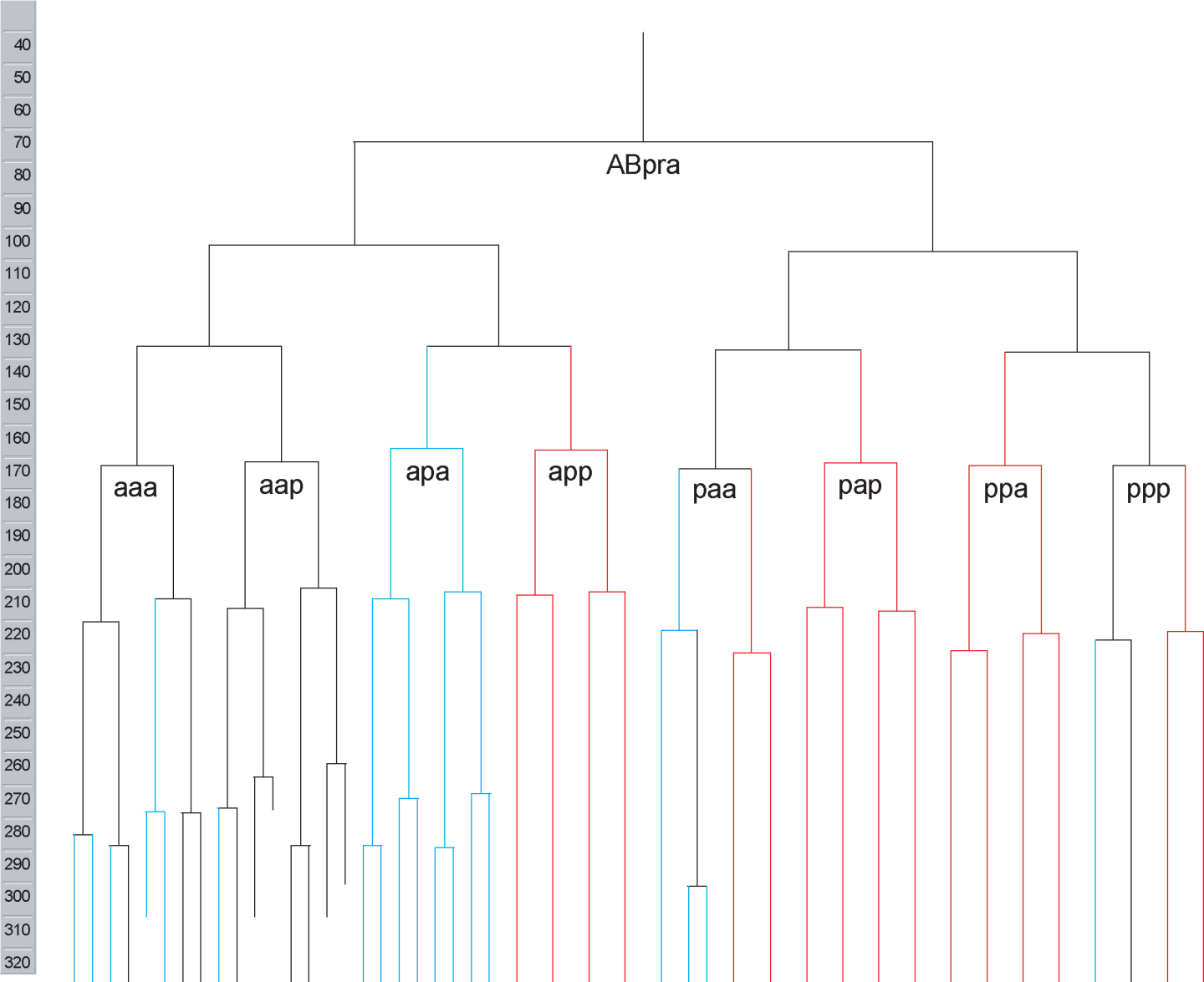
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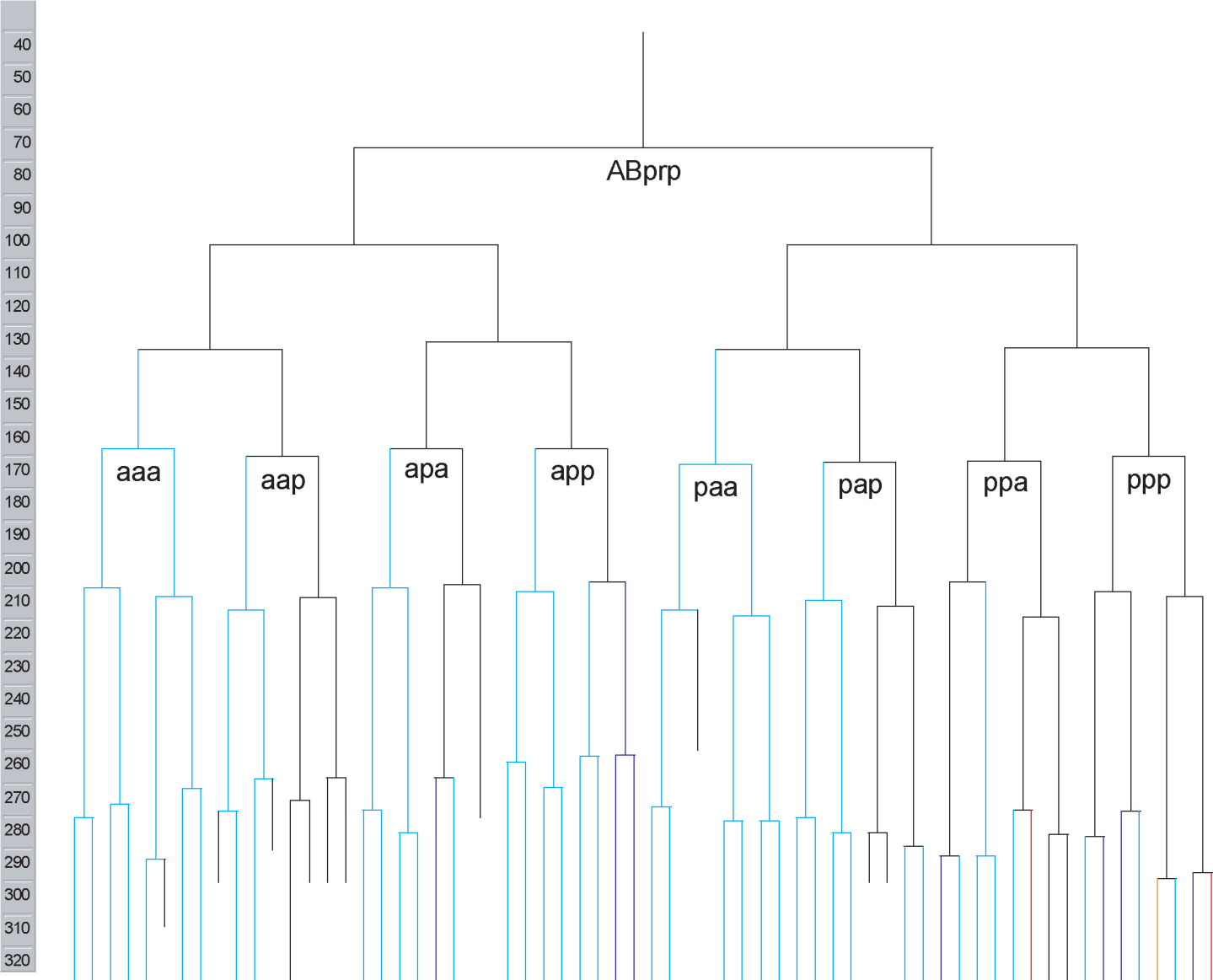
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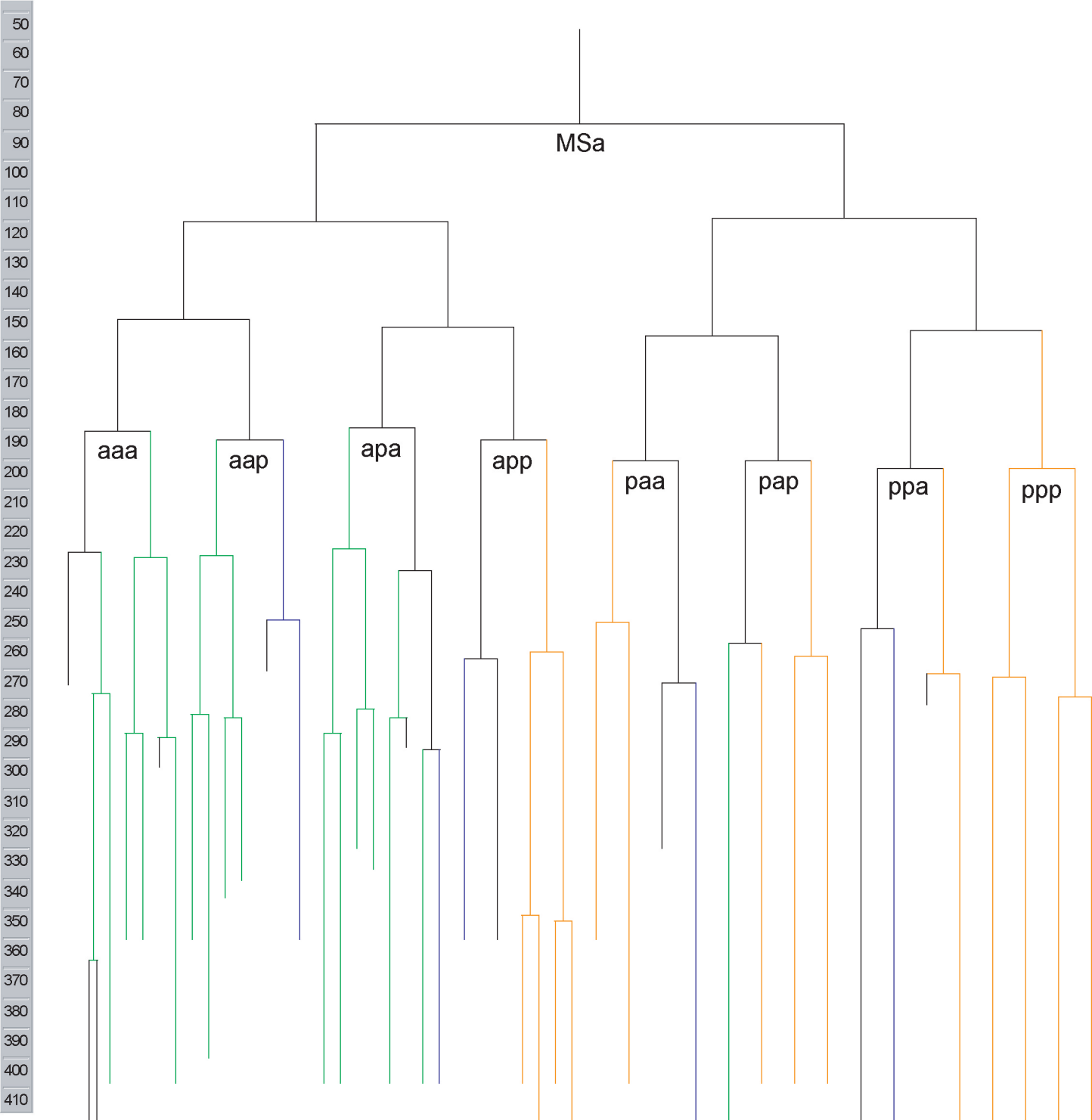
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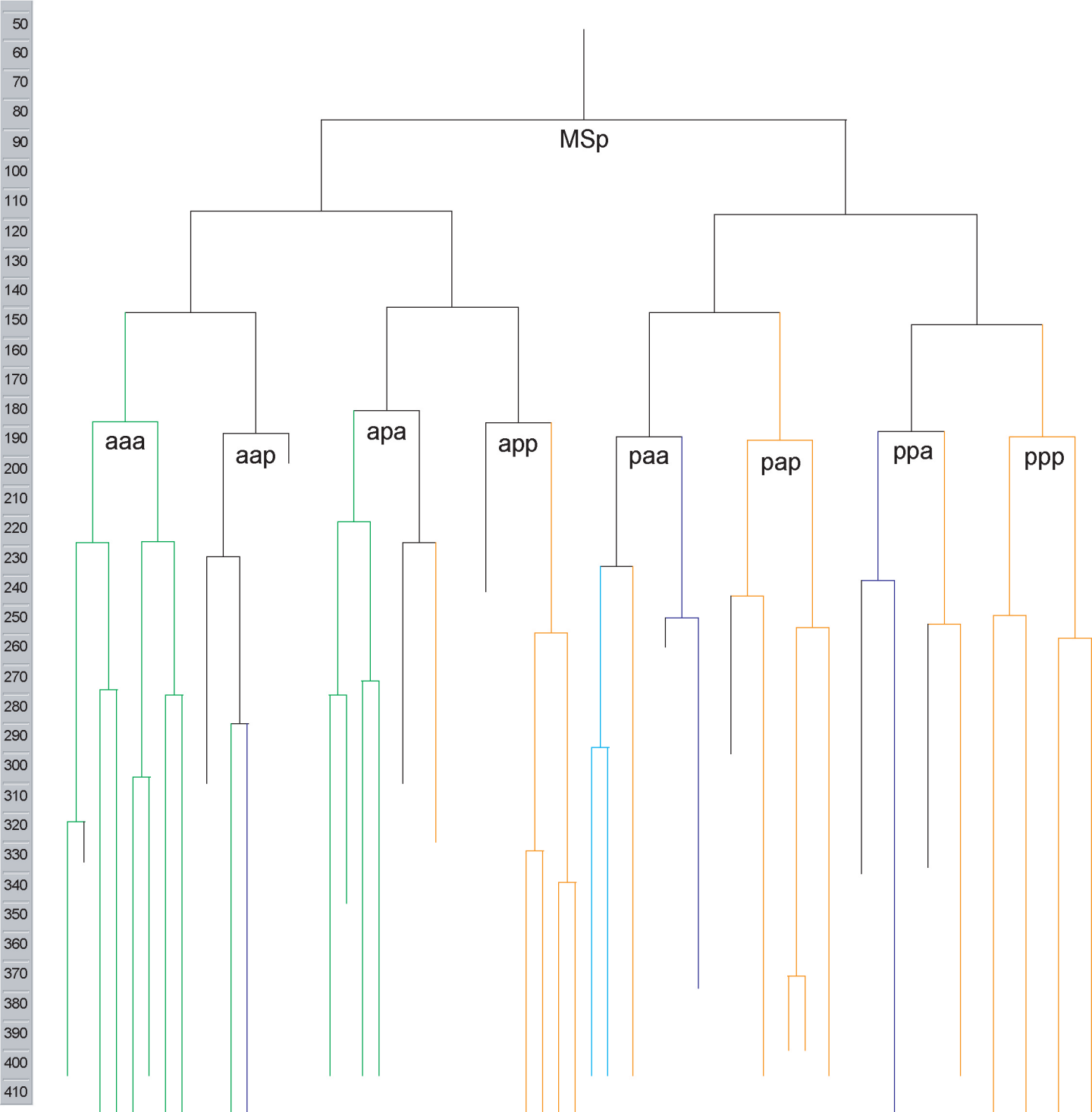
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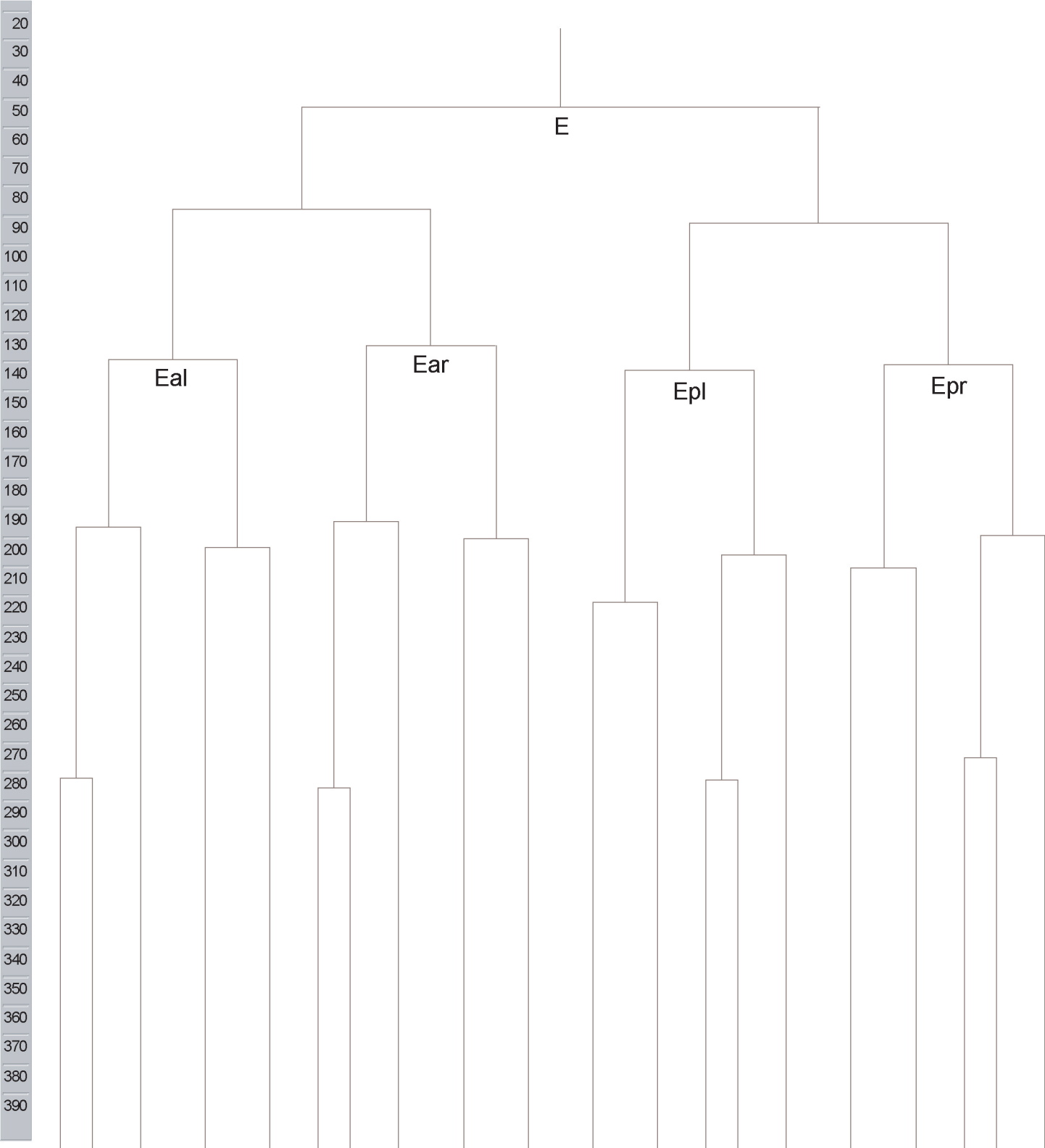
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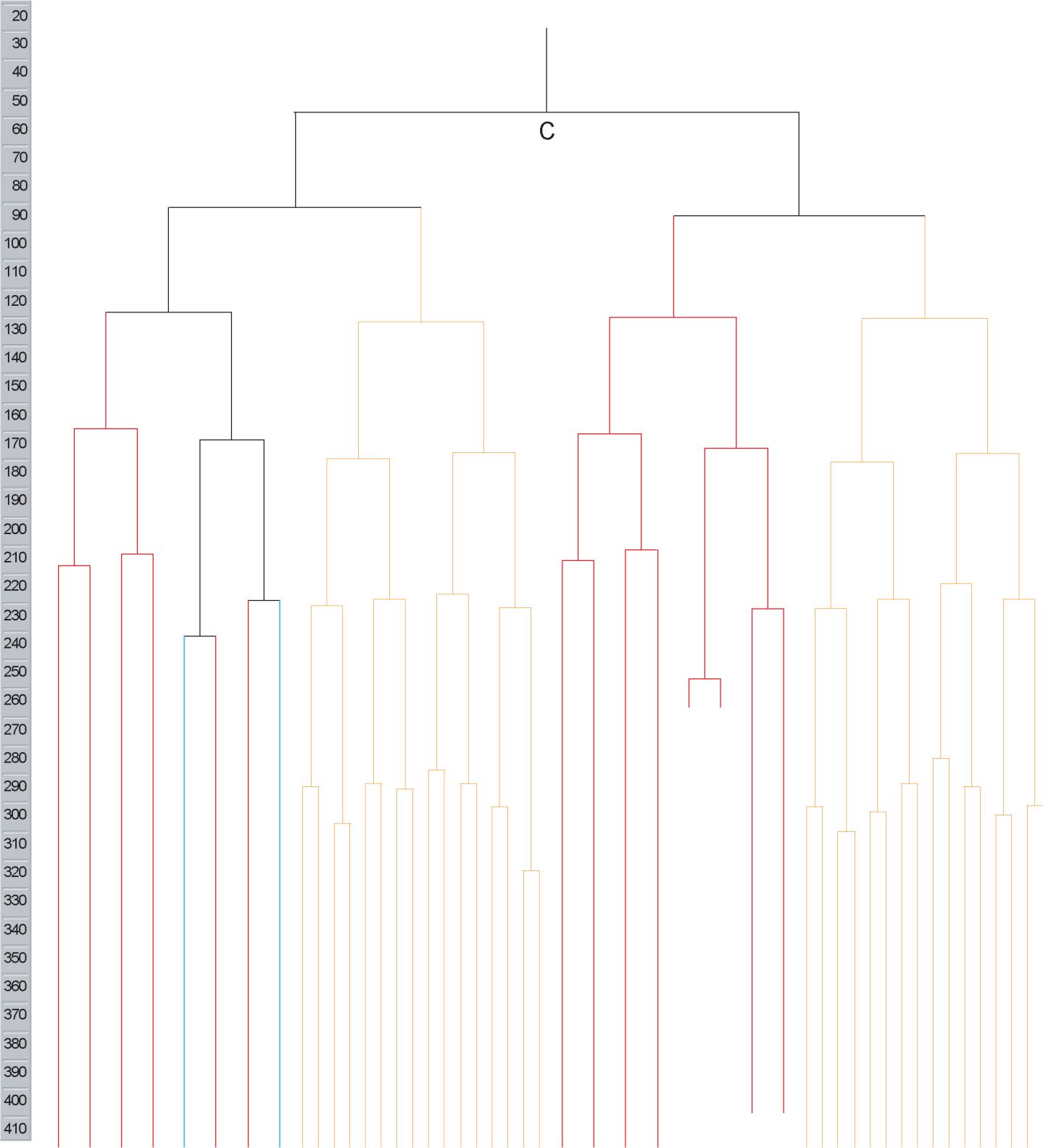
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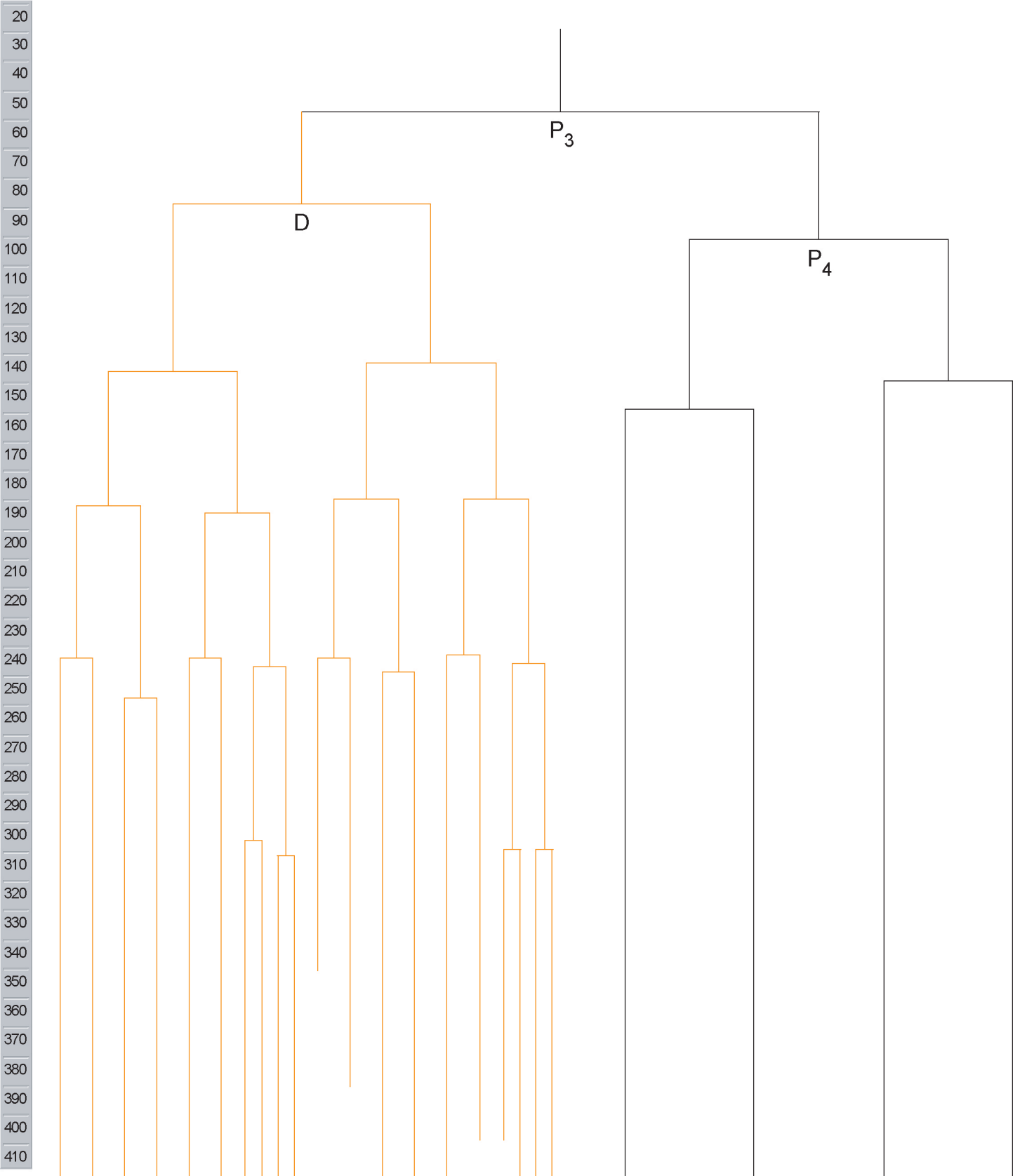
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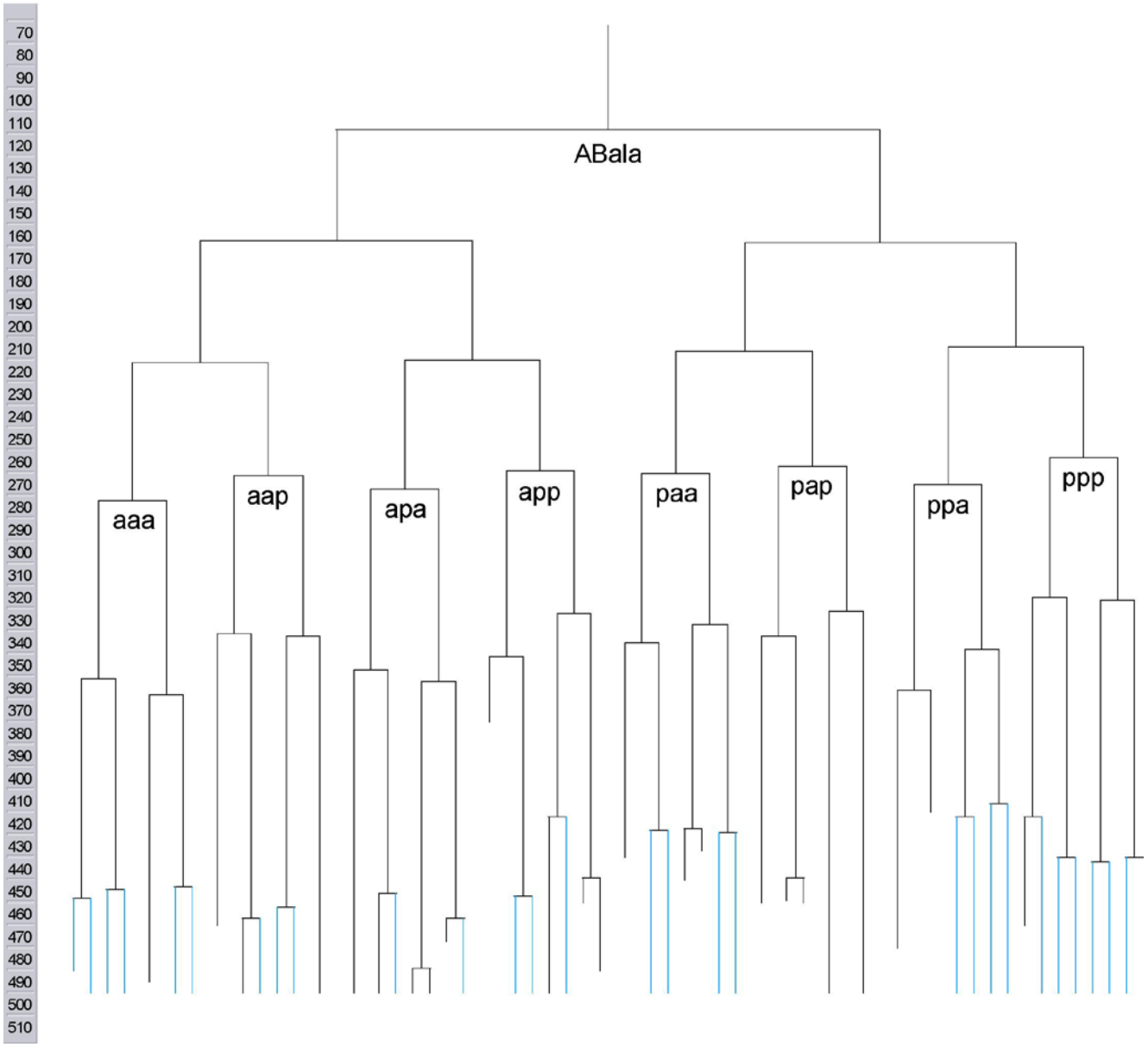
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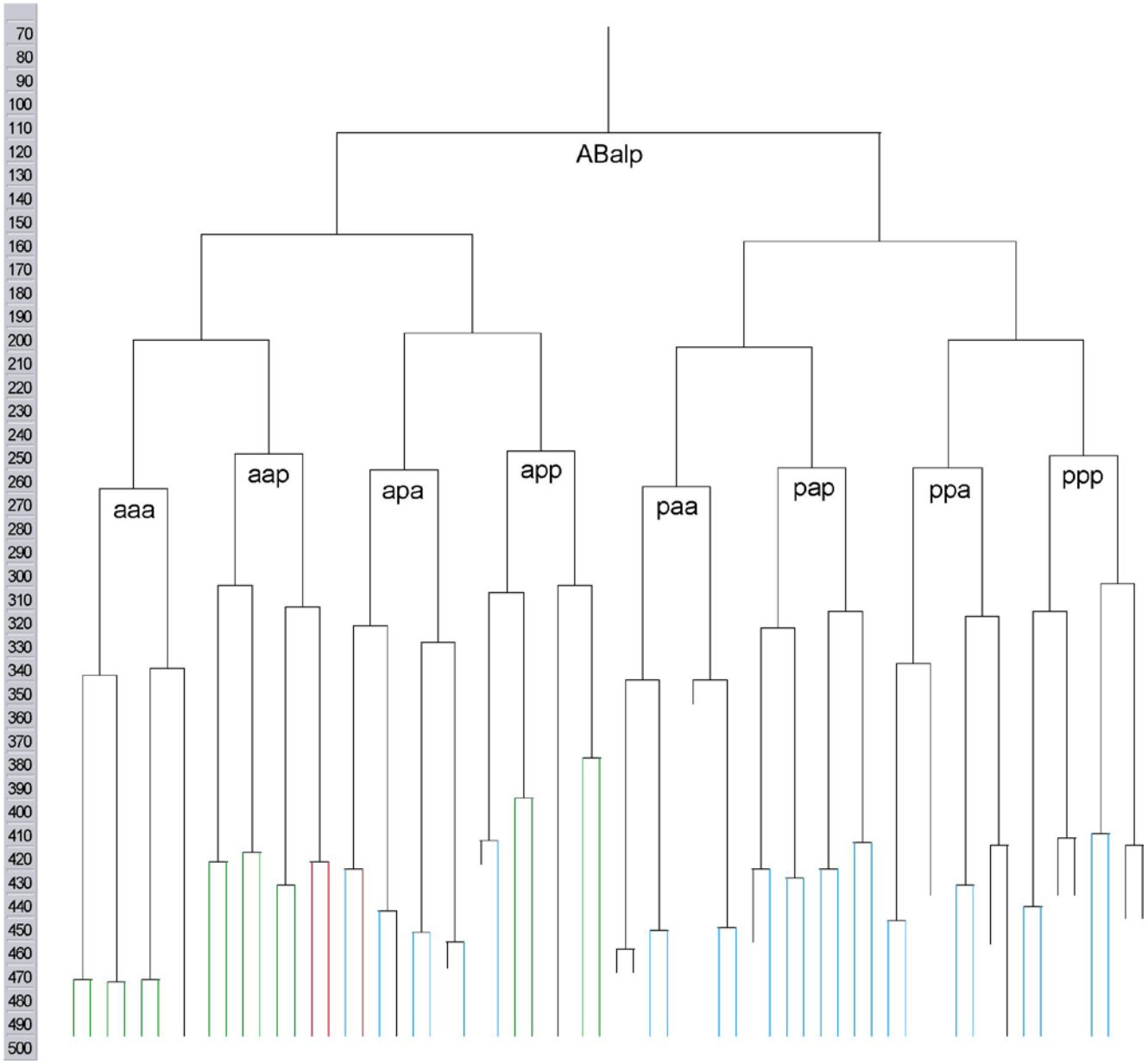
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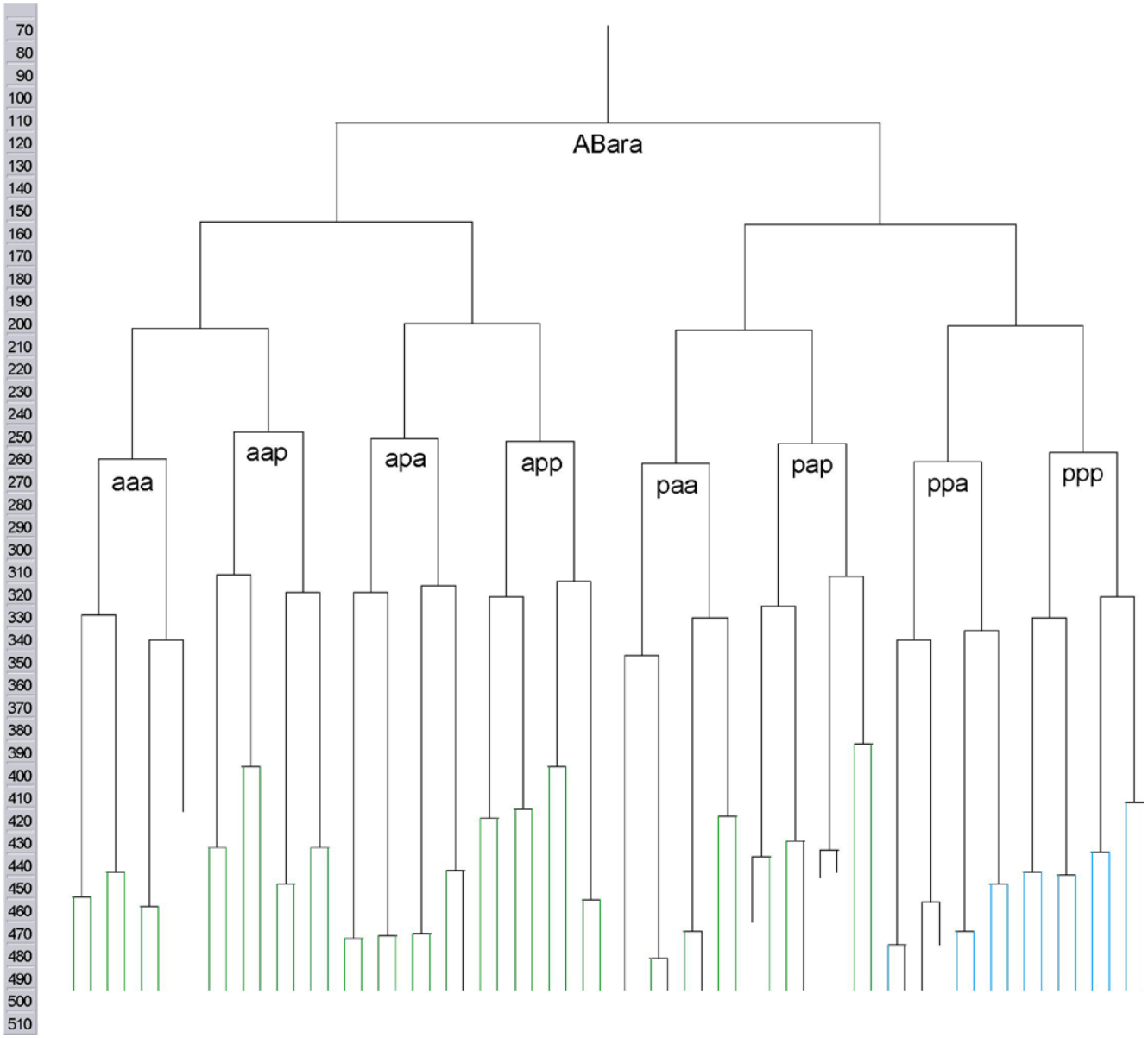
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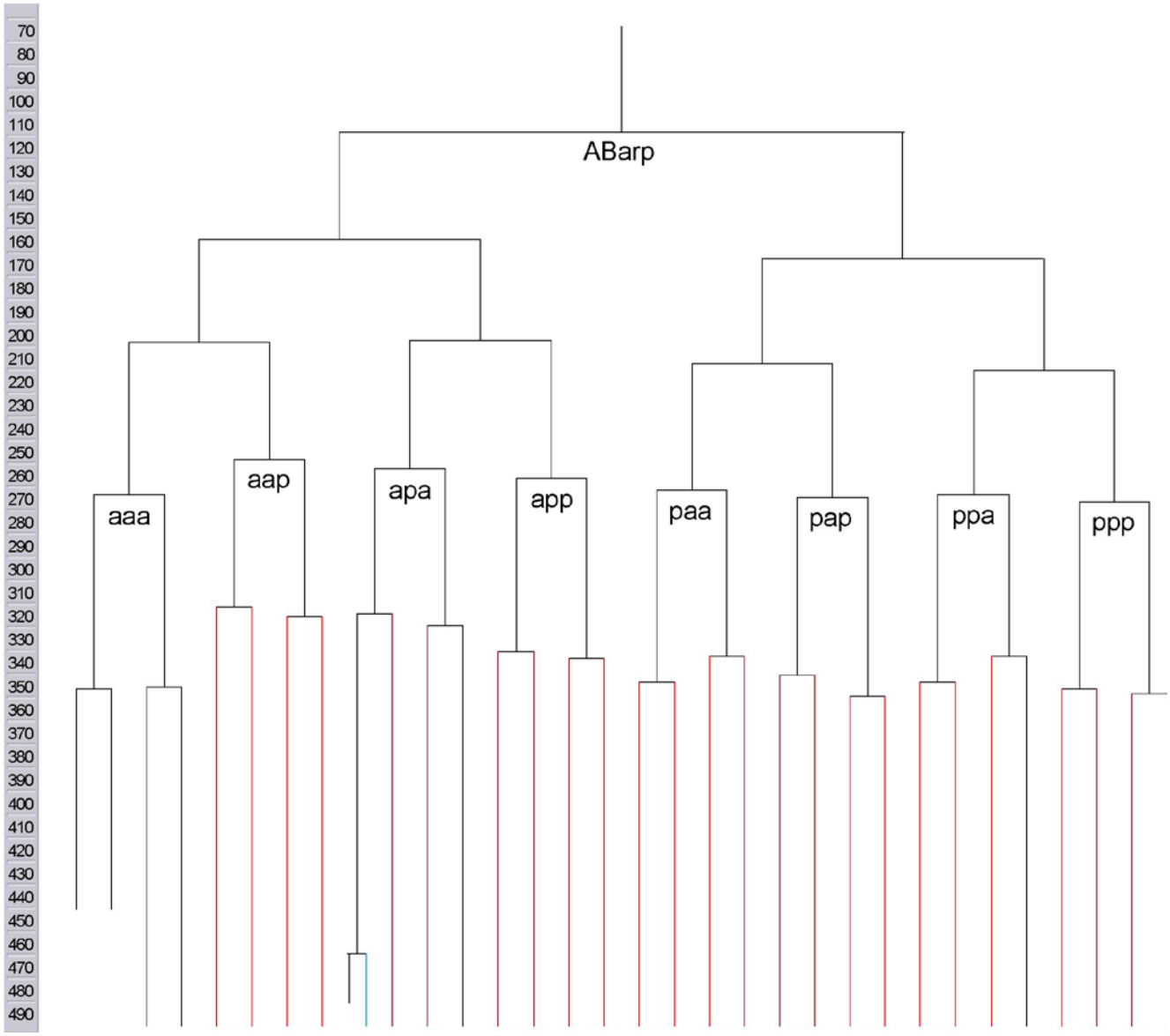
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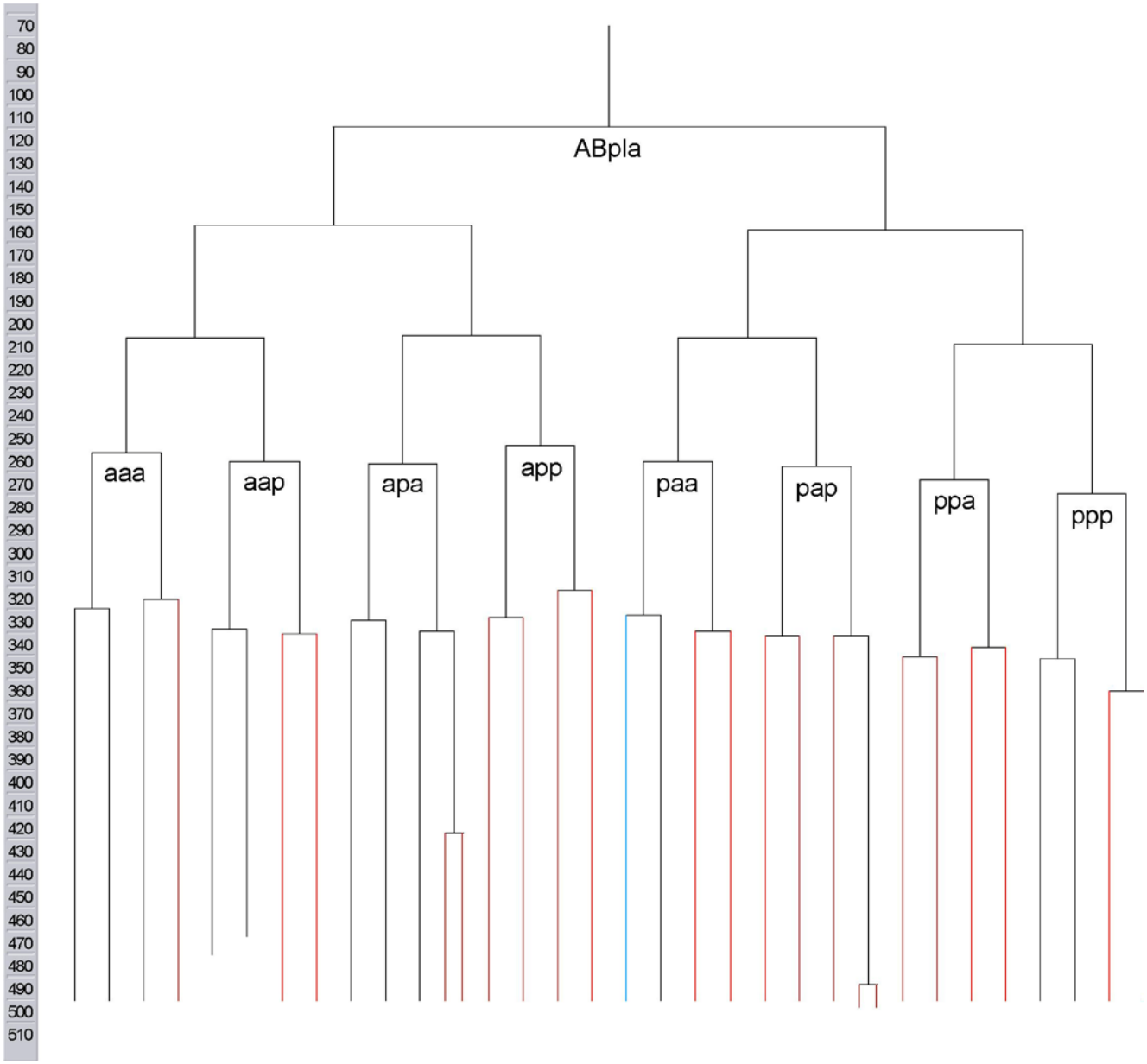
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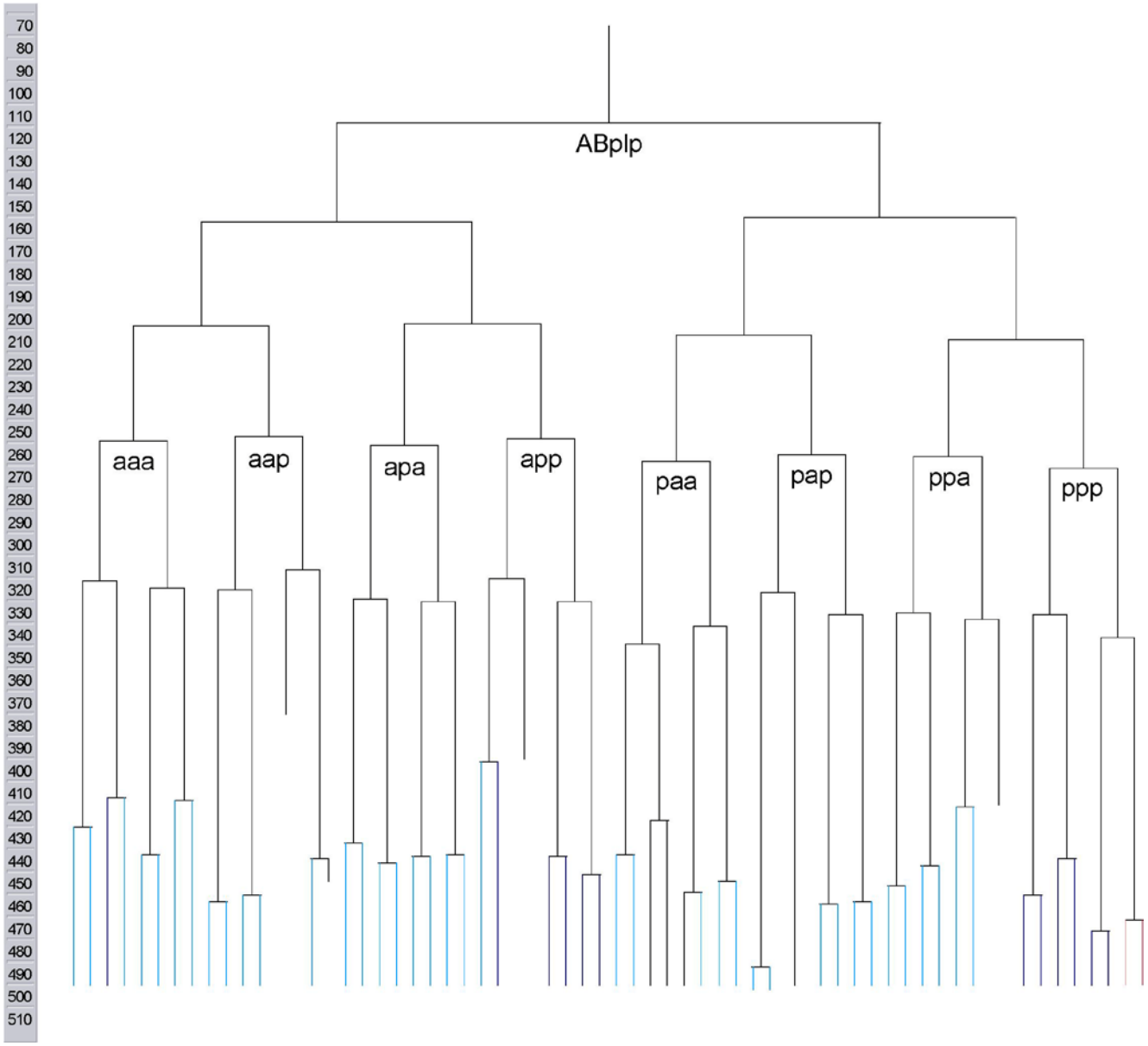
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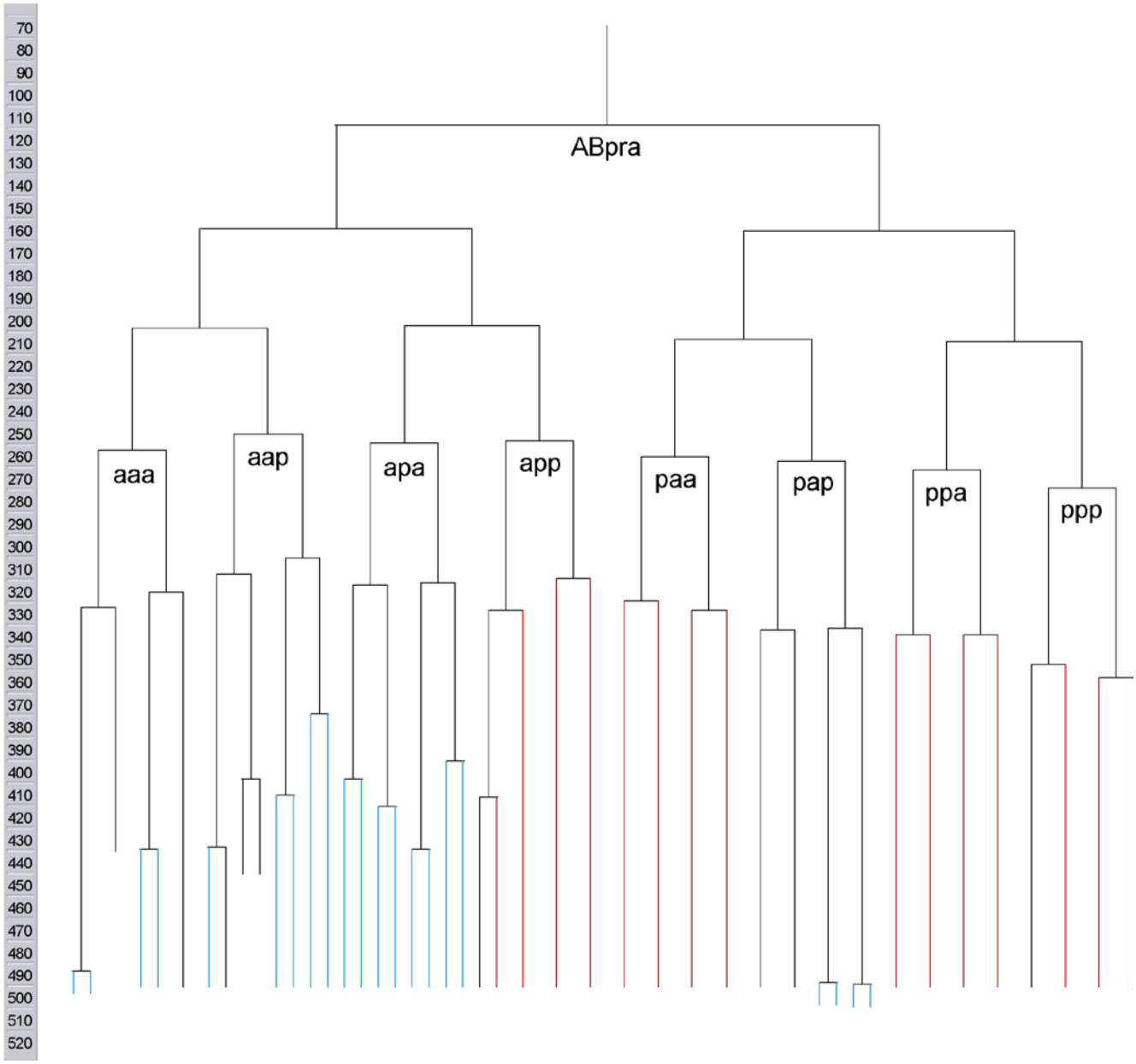
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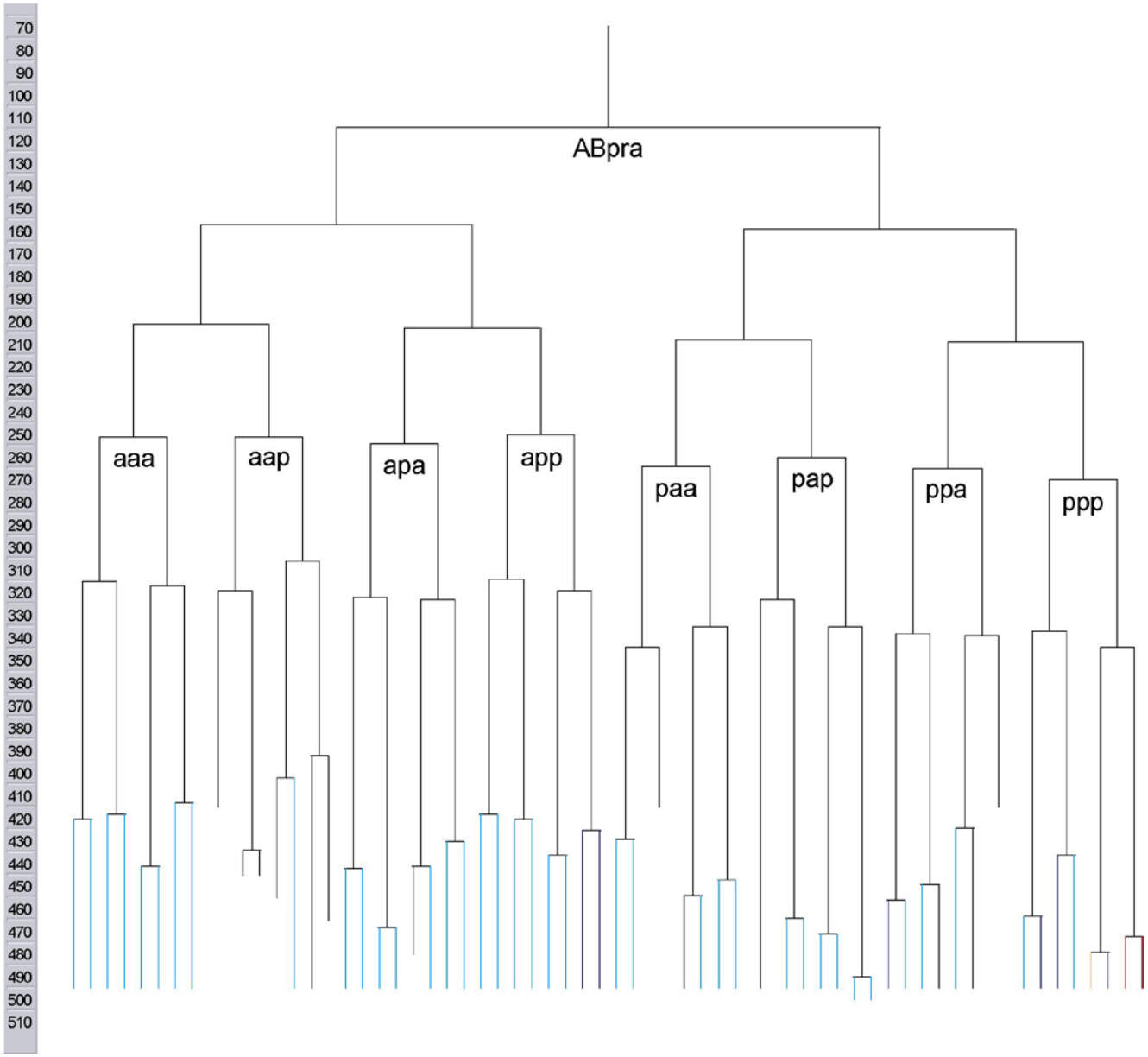
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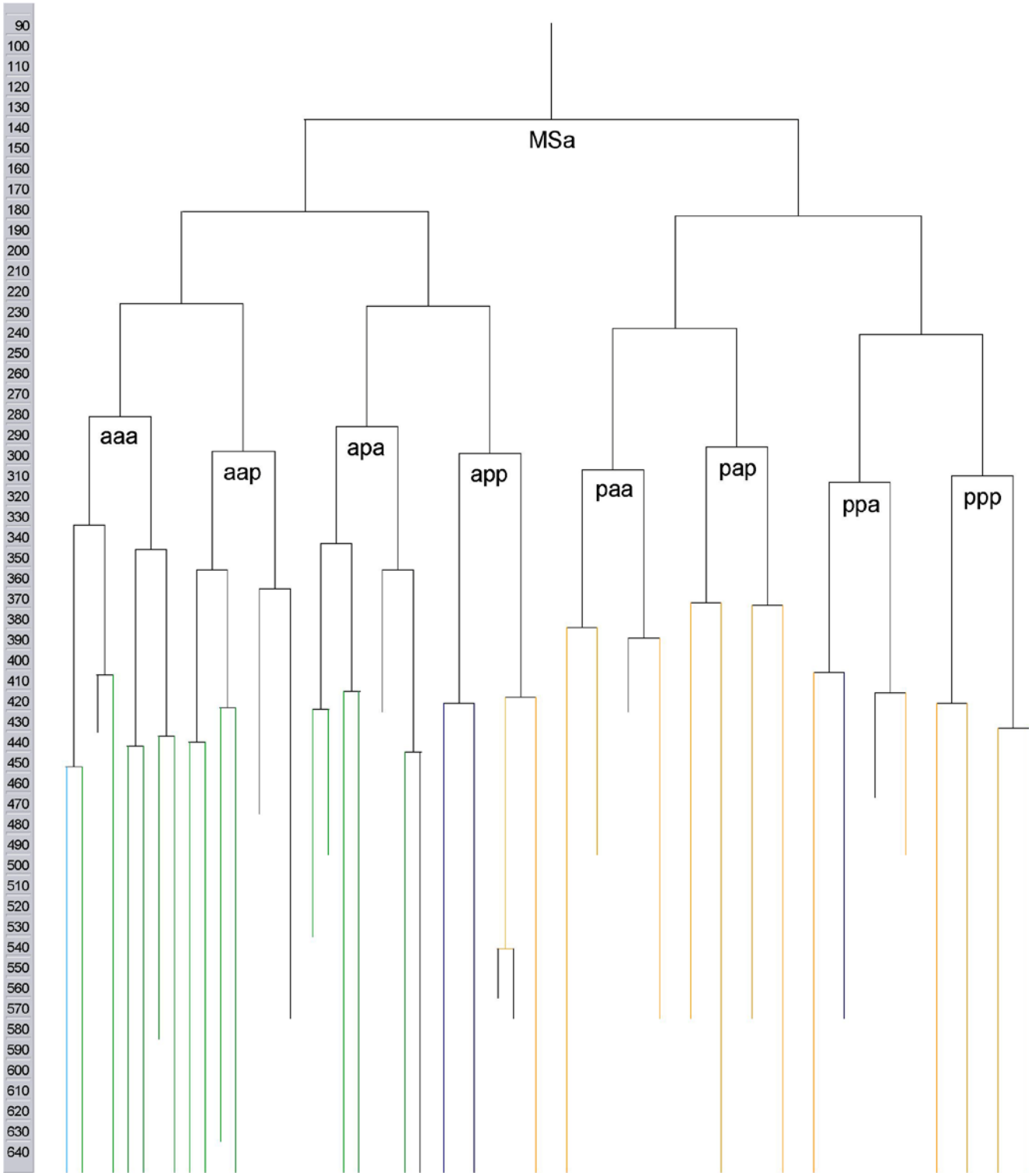
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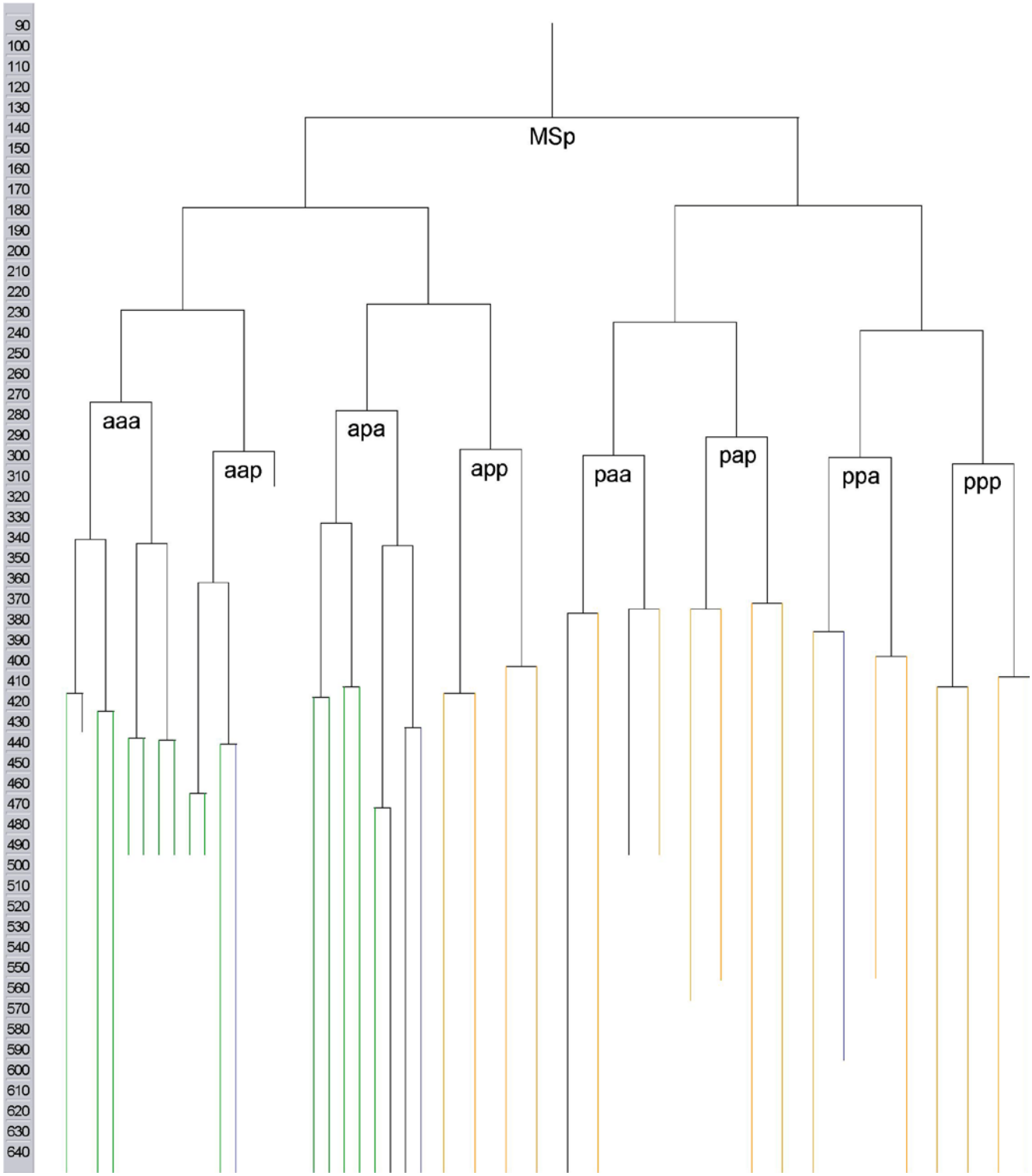
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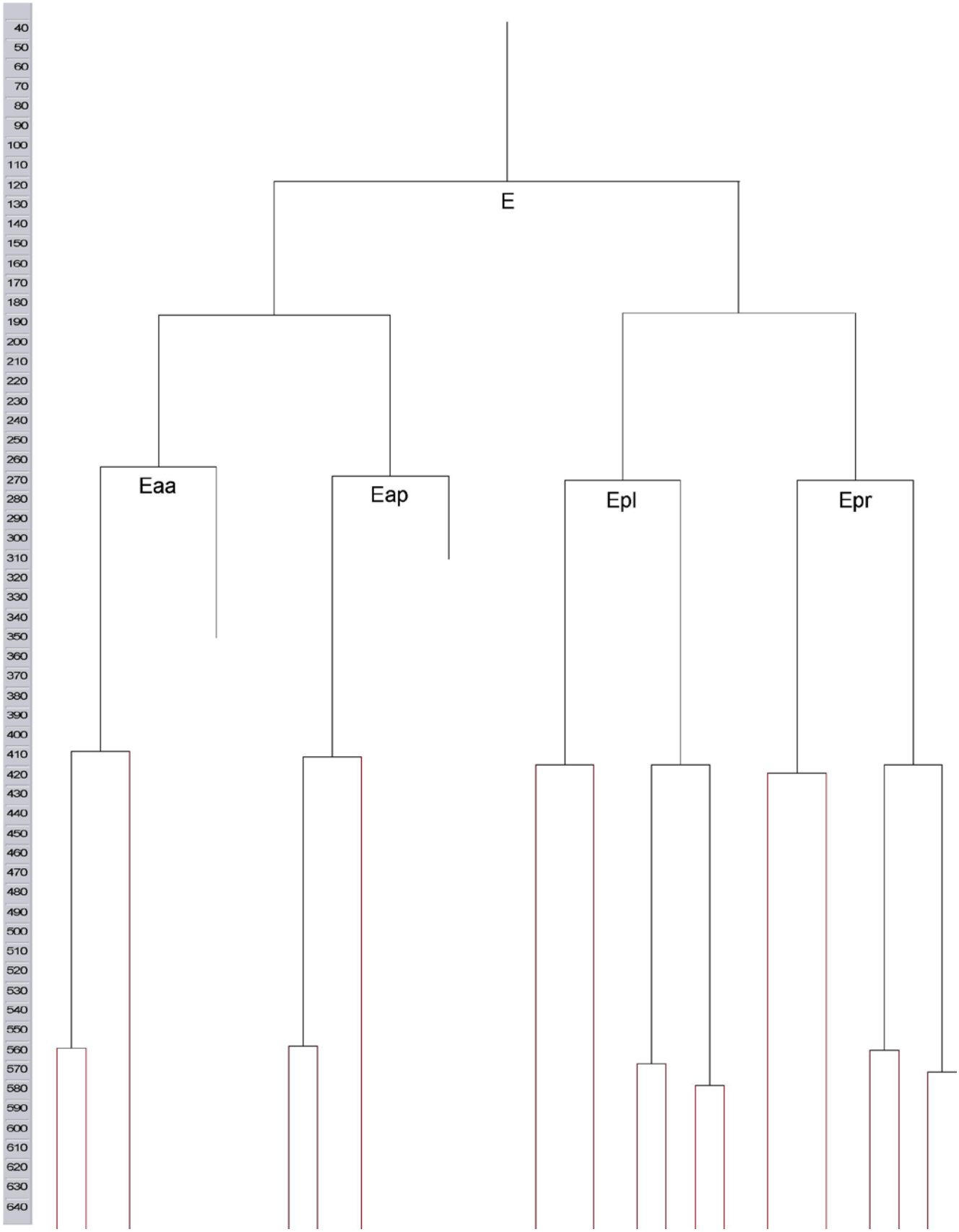
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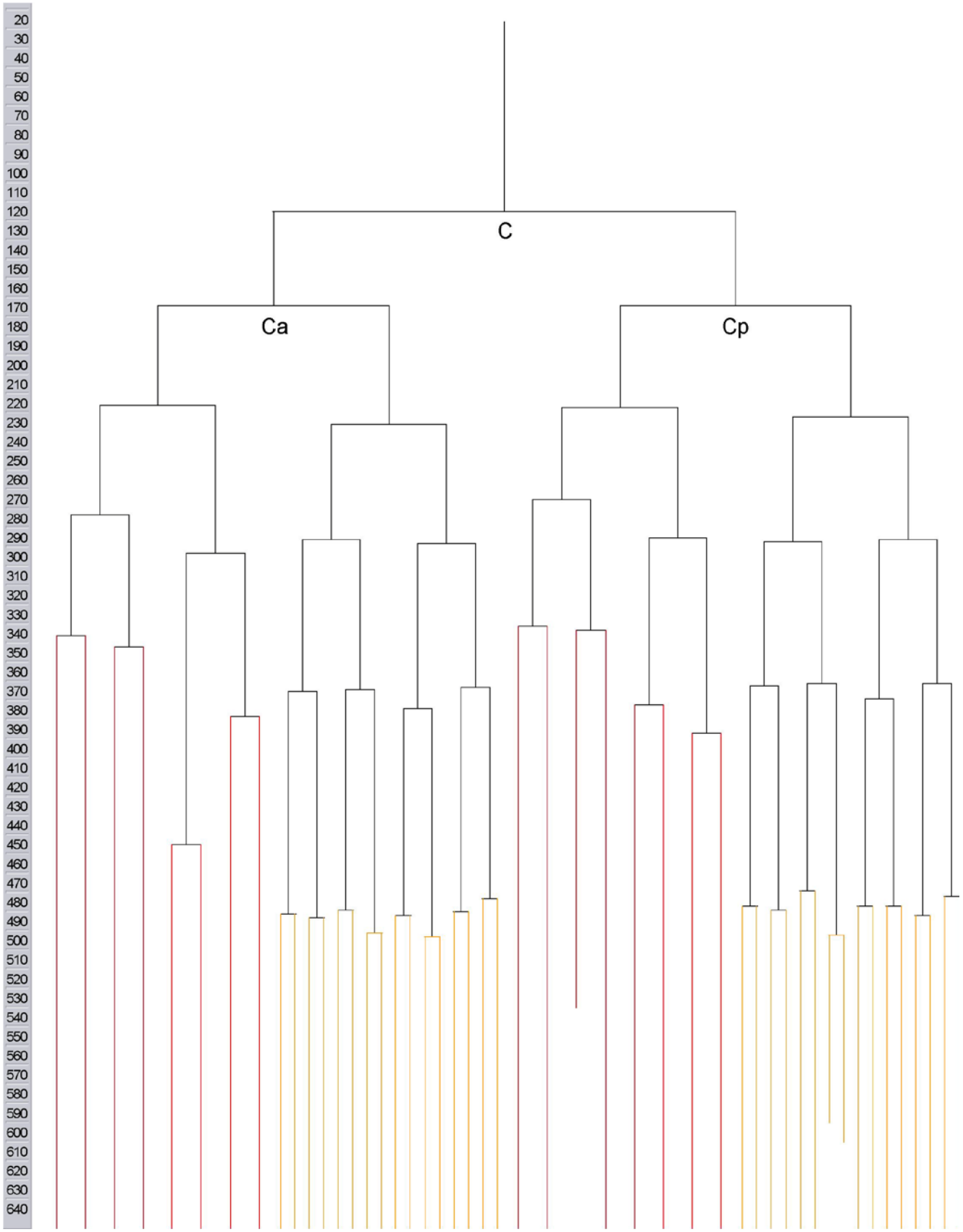
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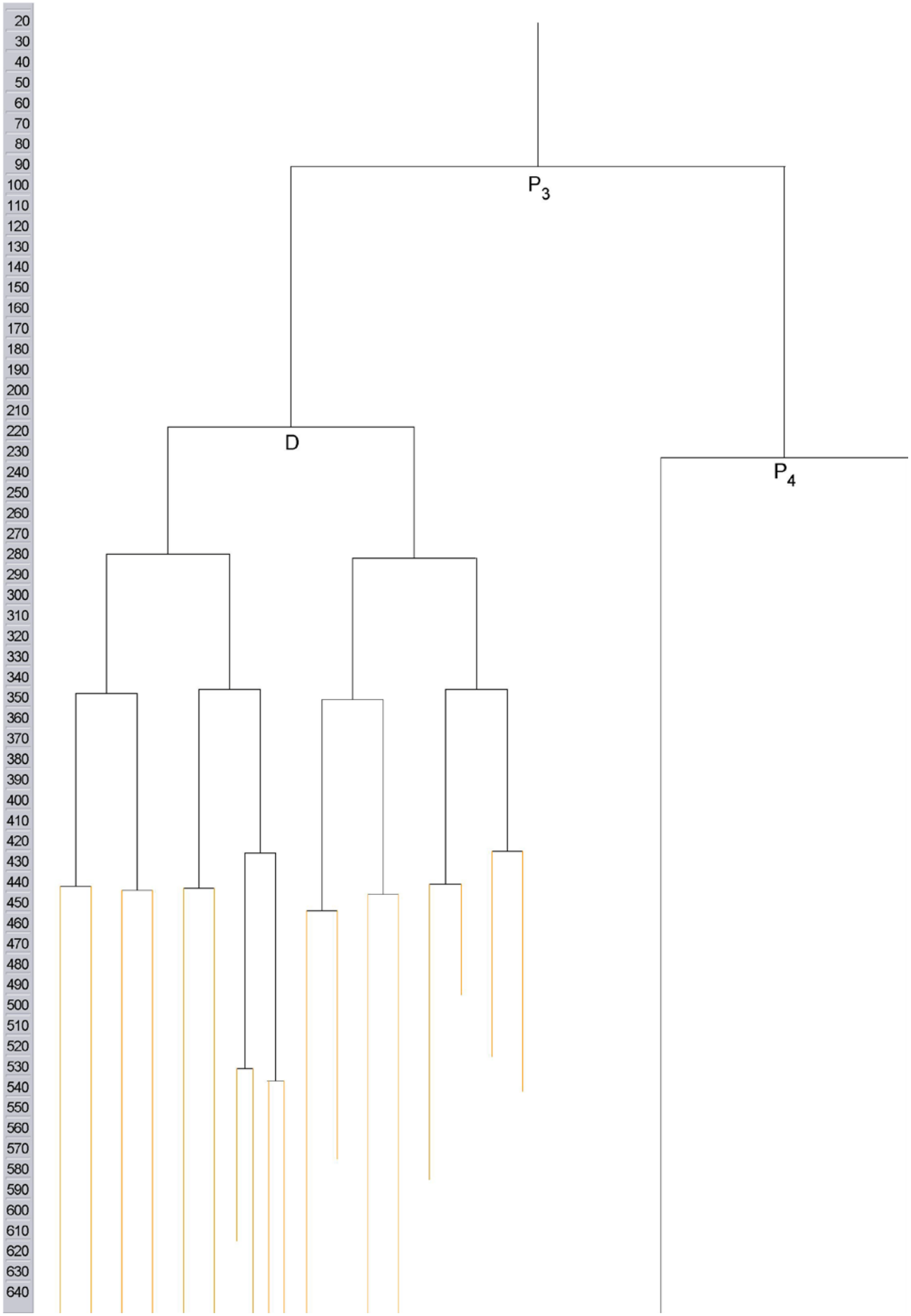
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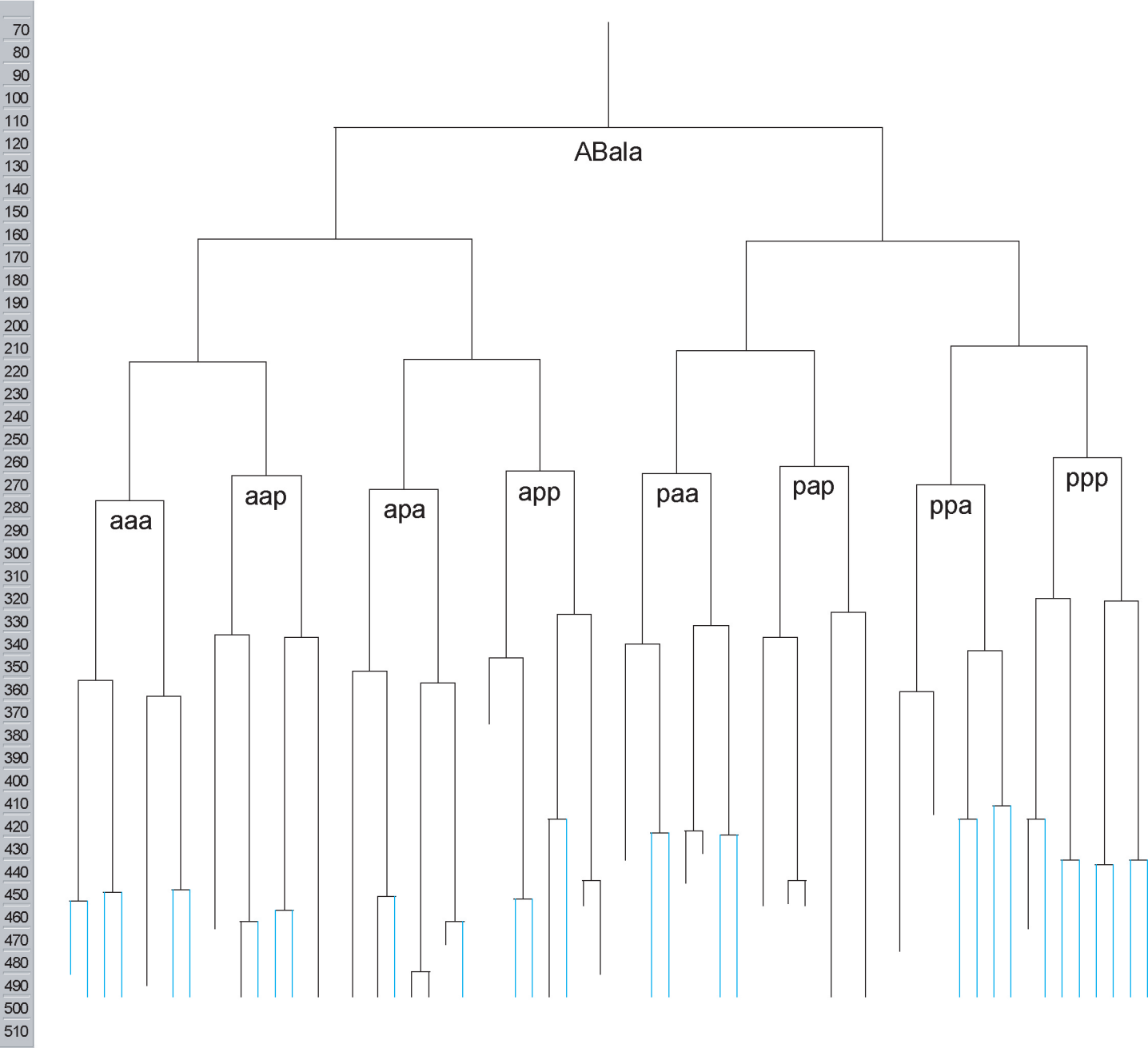
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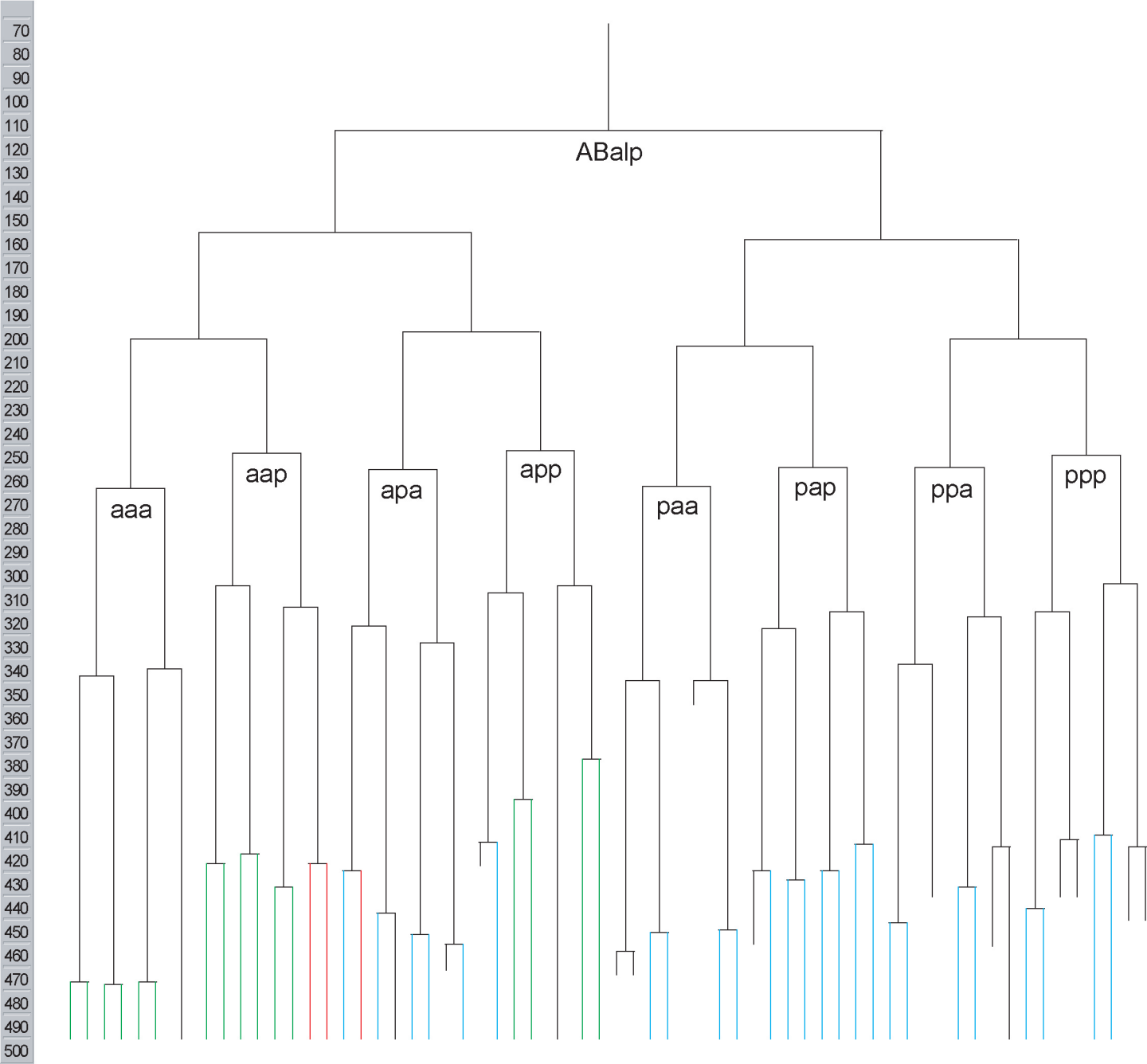
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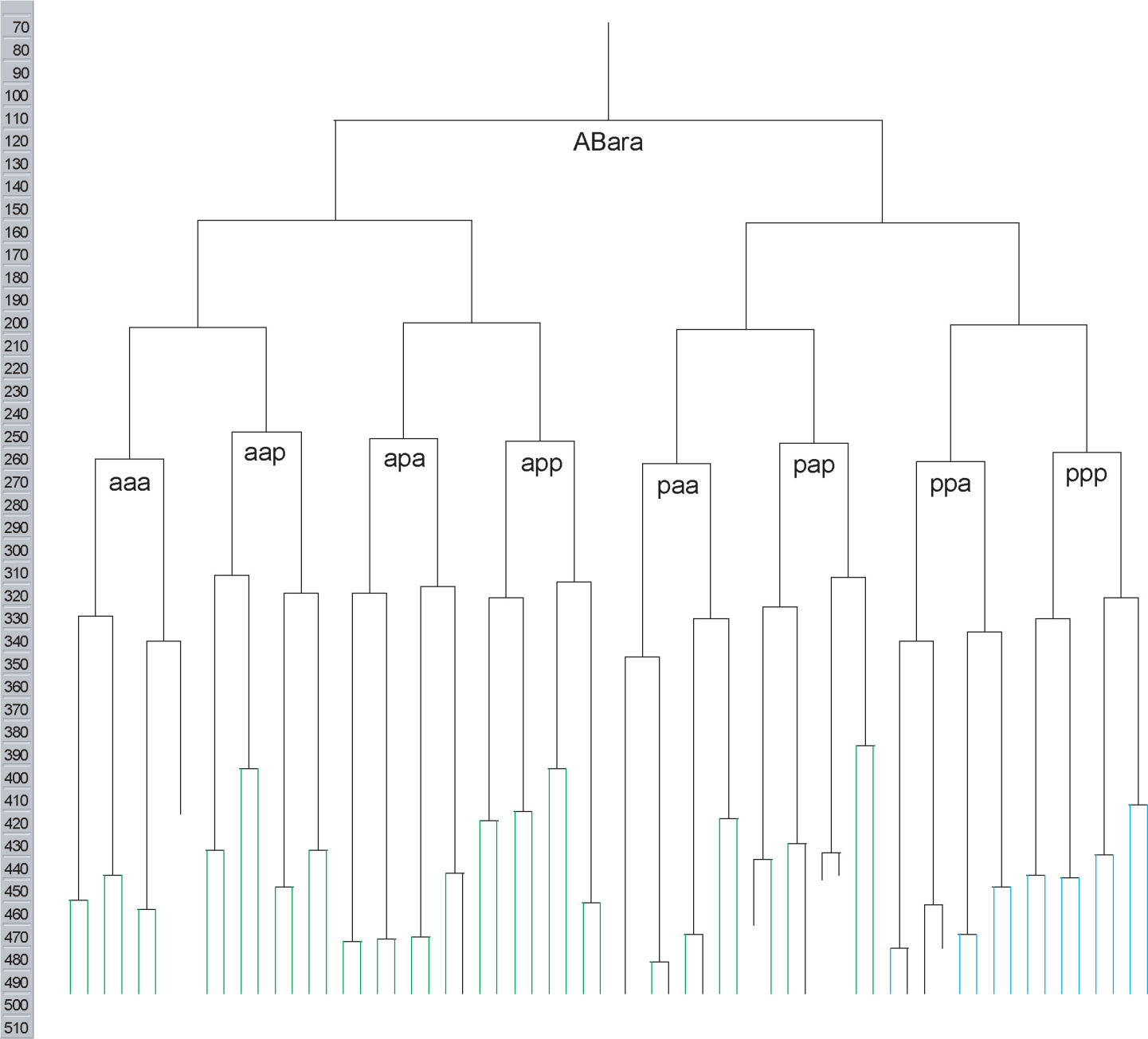
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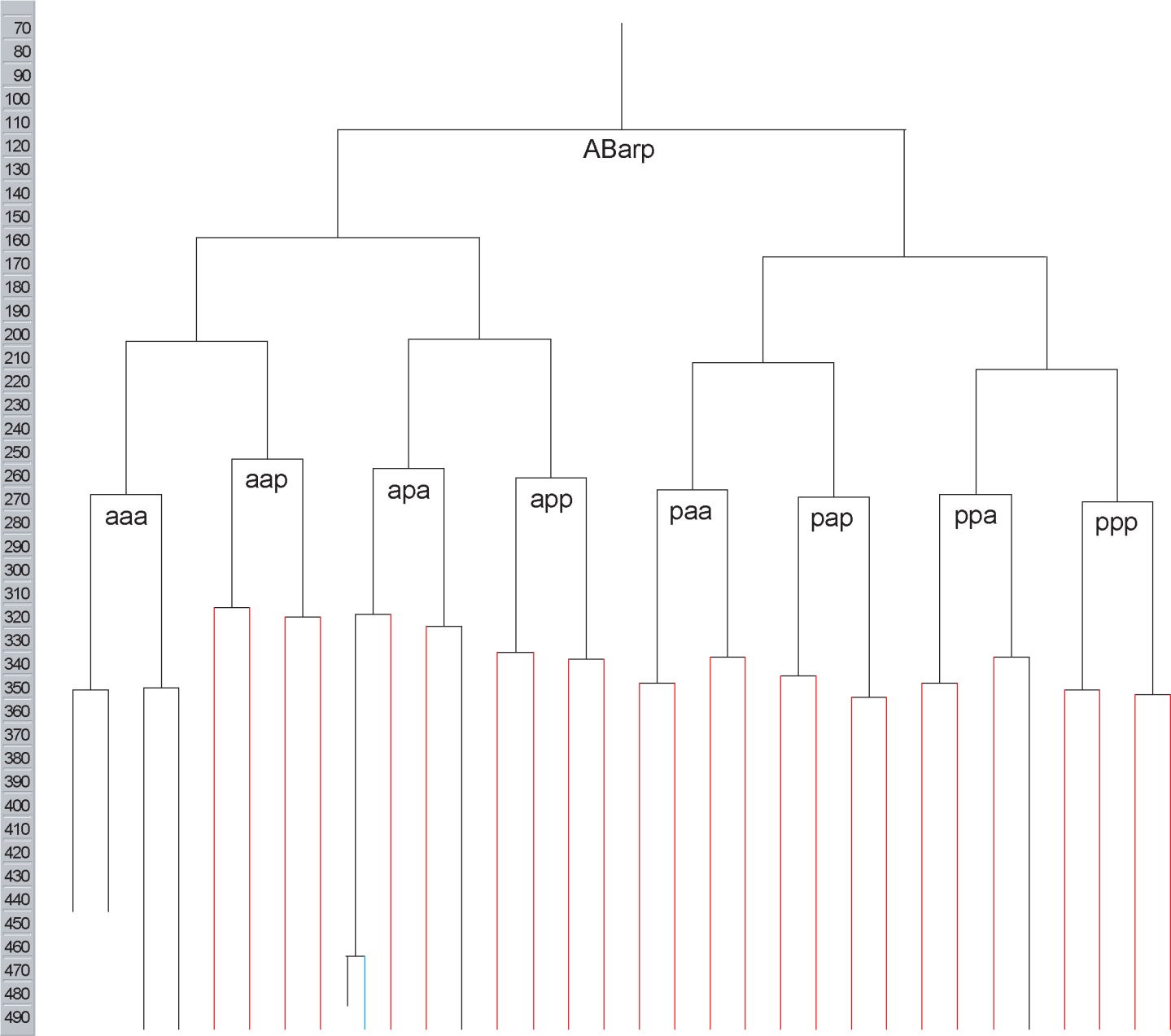
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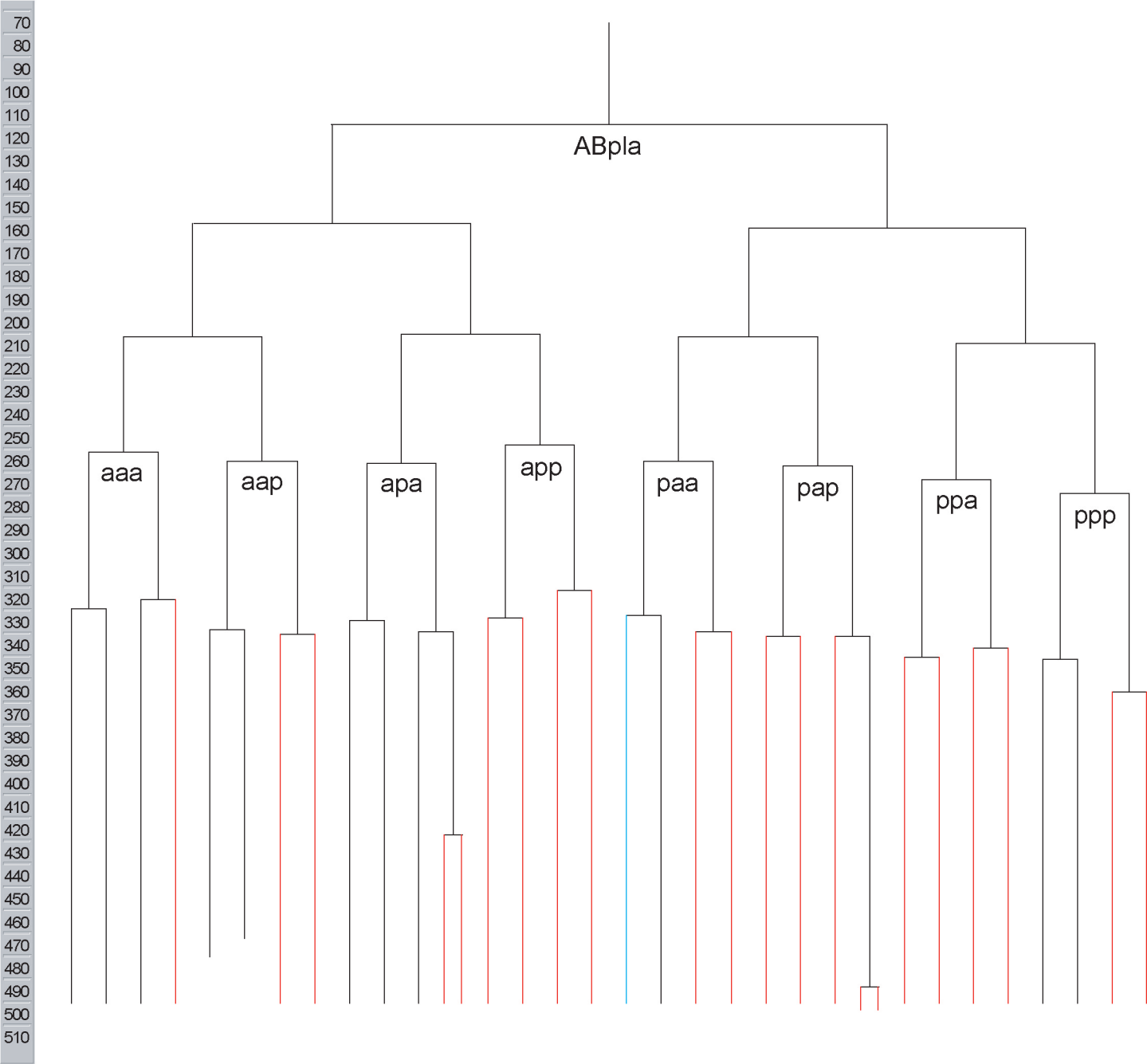
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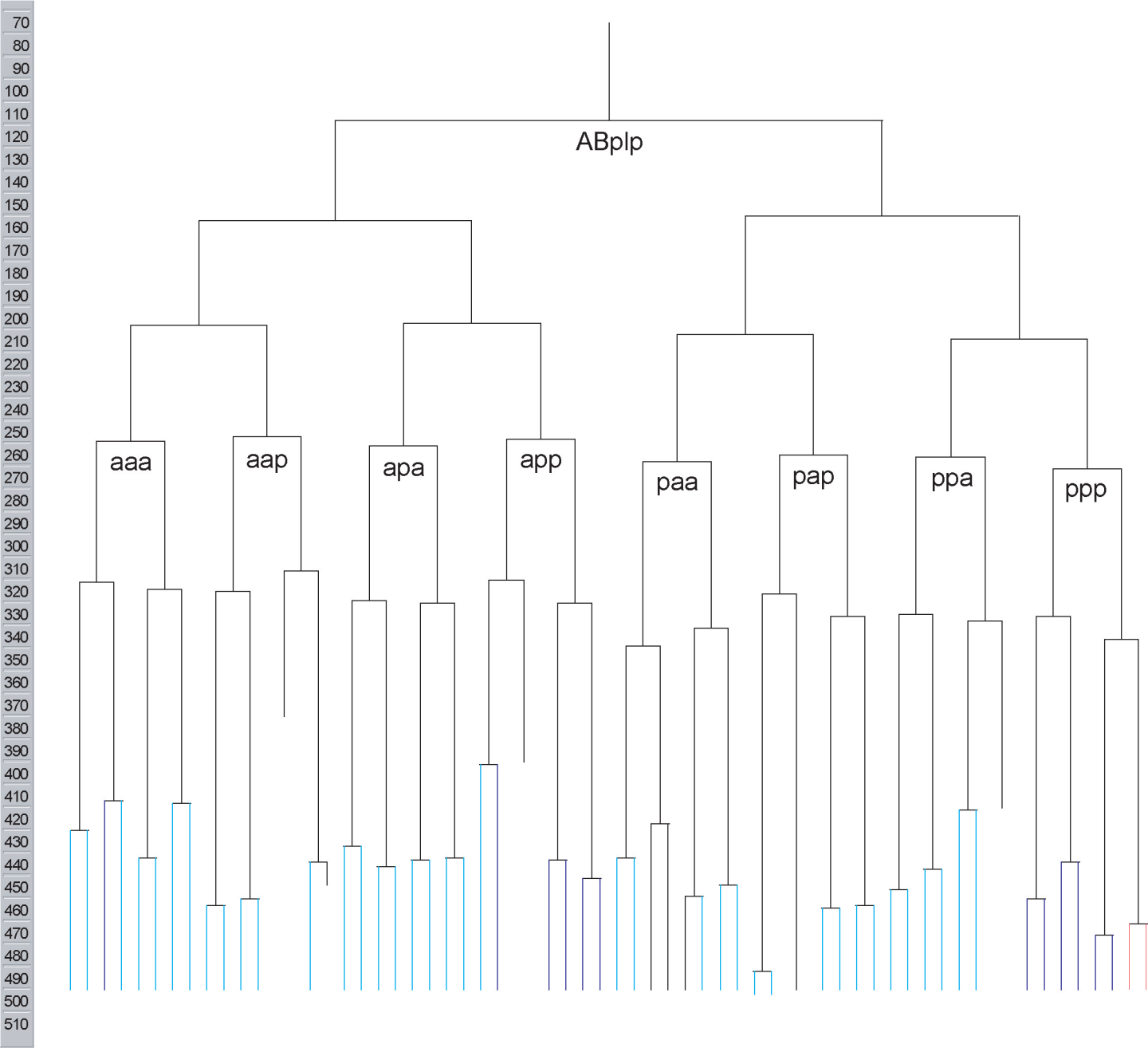
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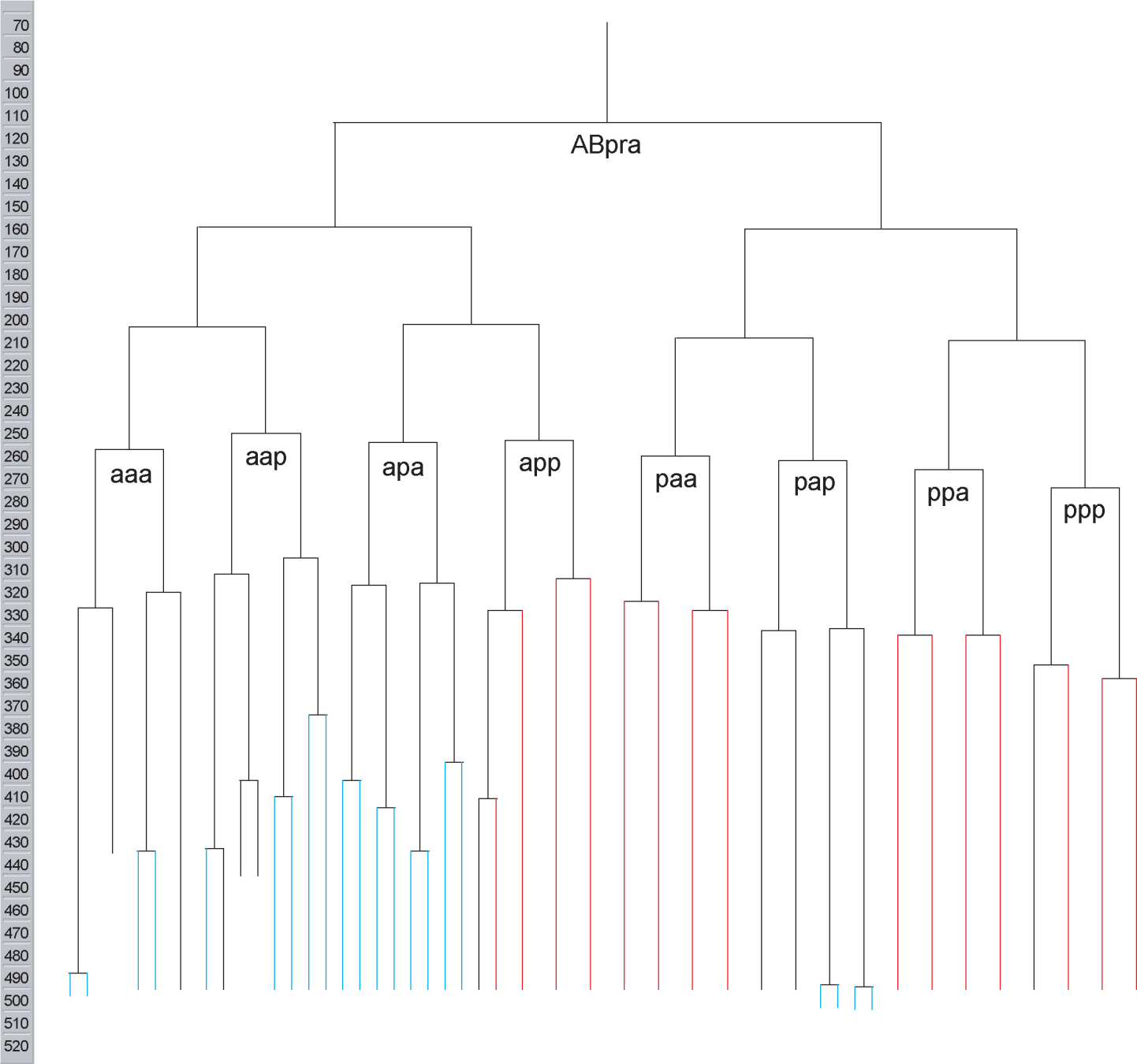
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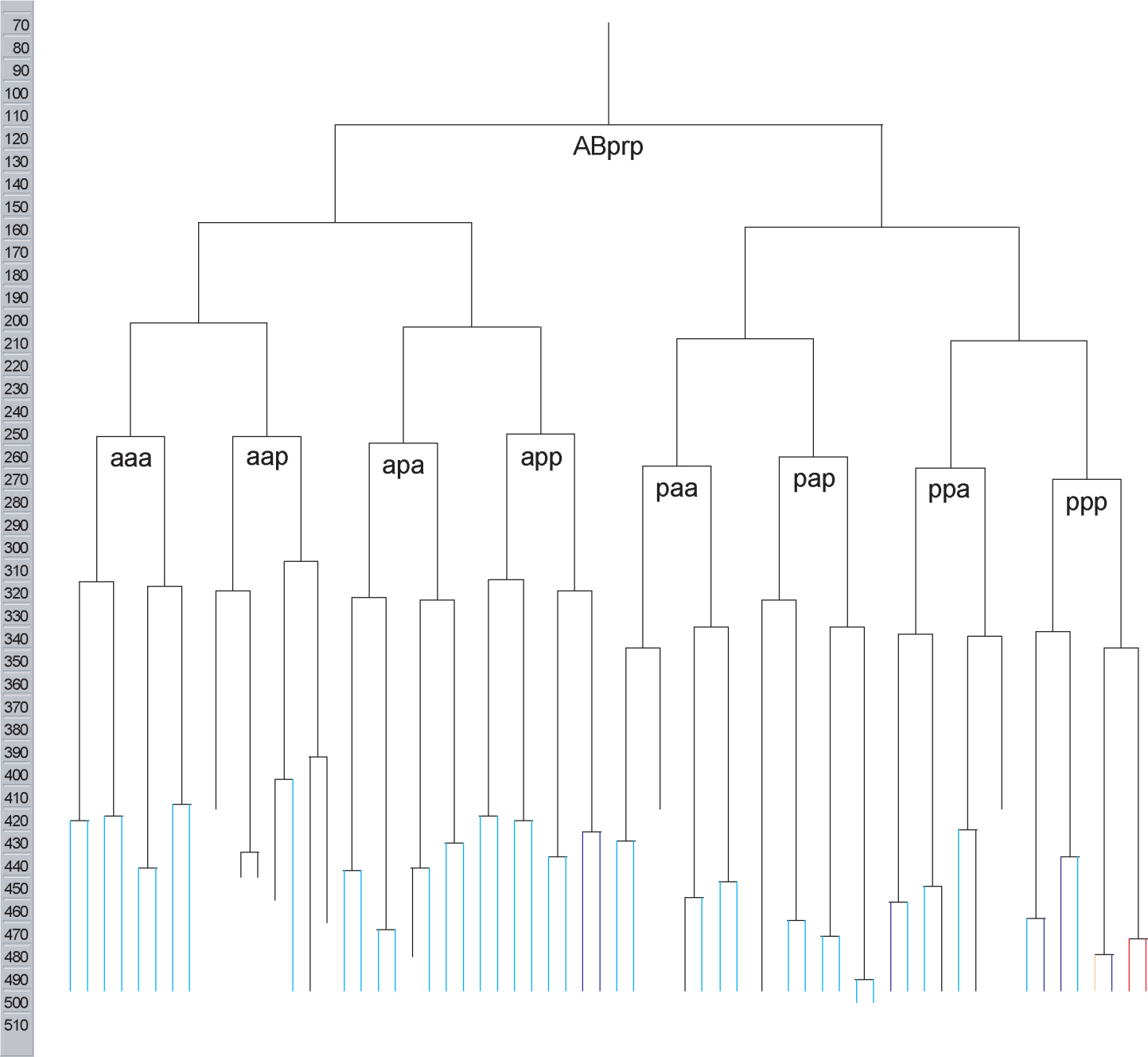
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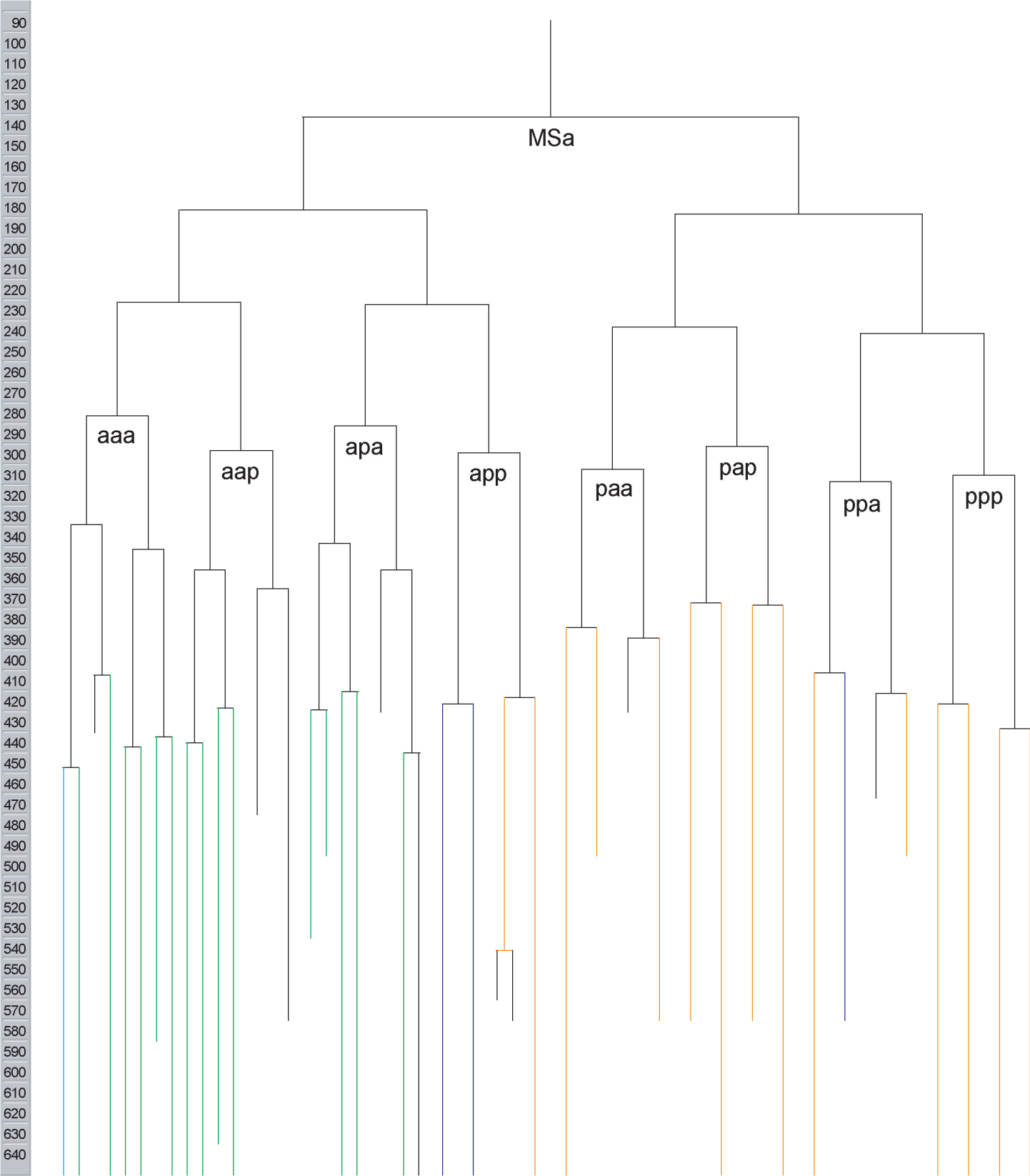
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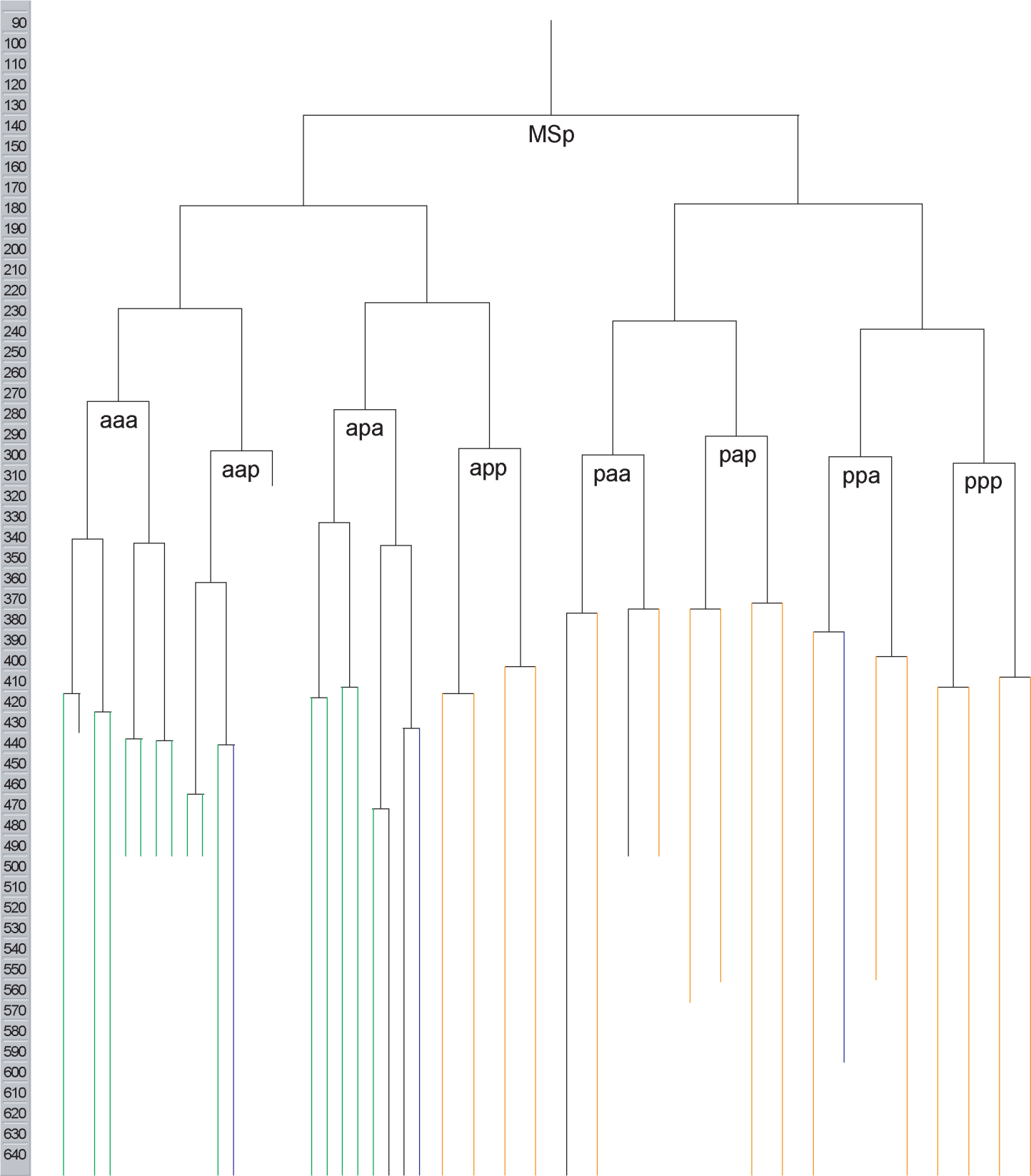
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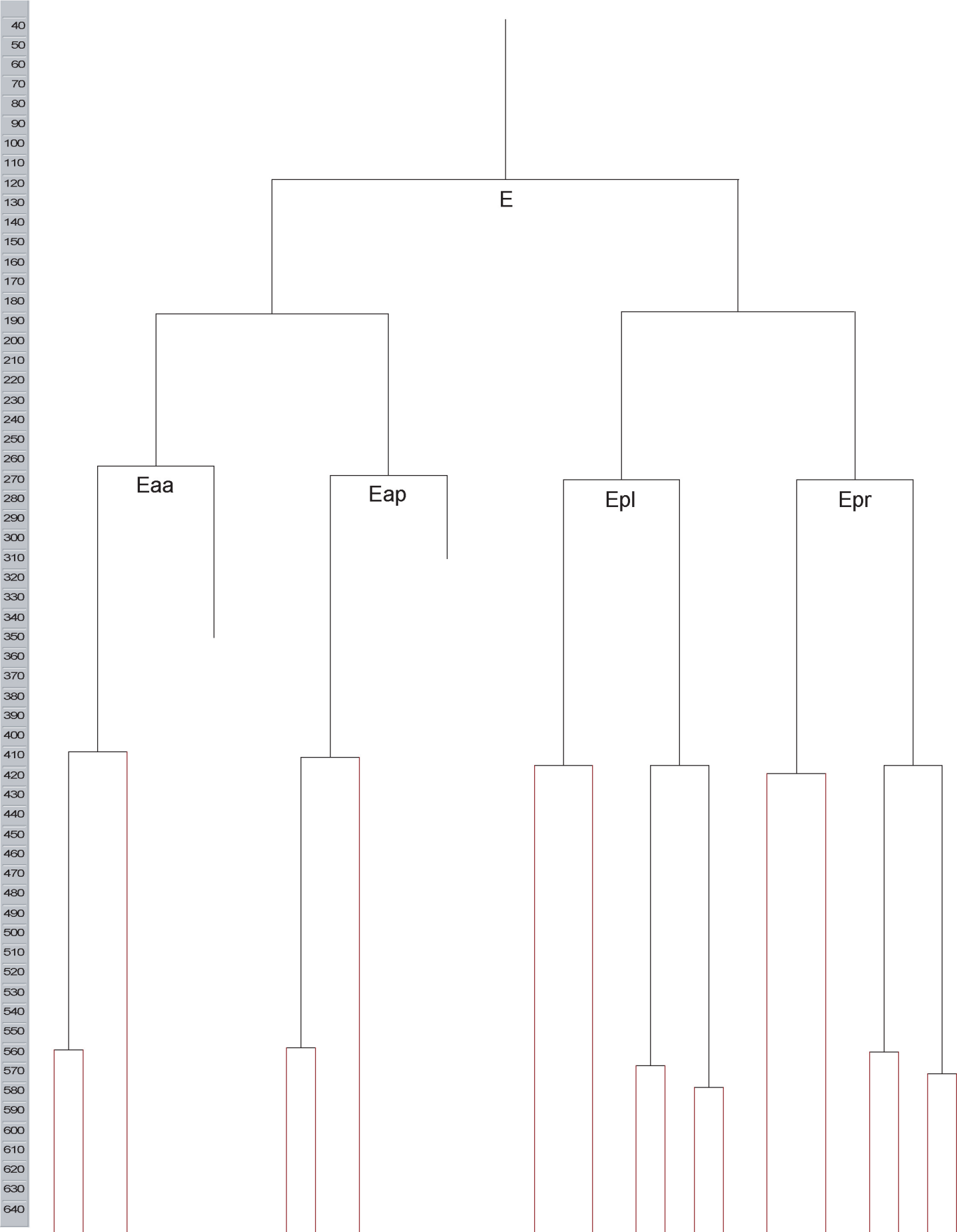
ppp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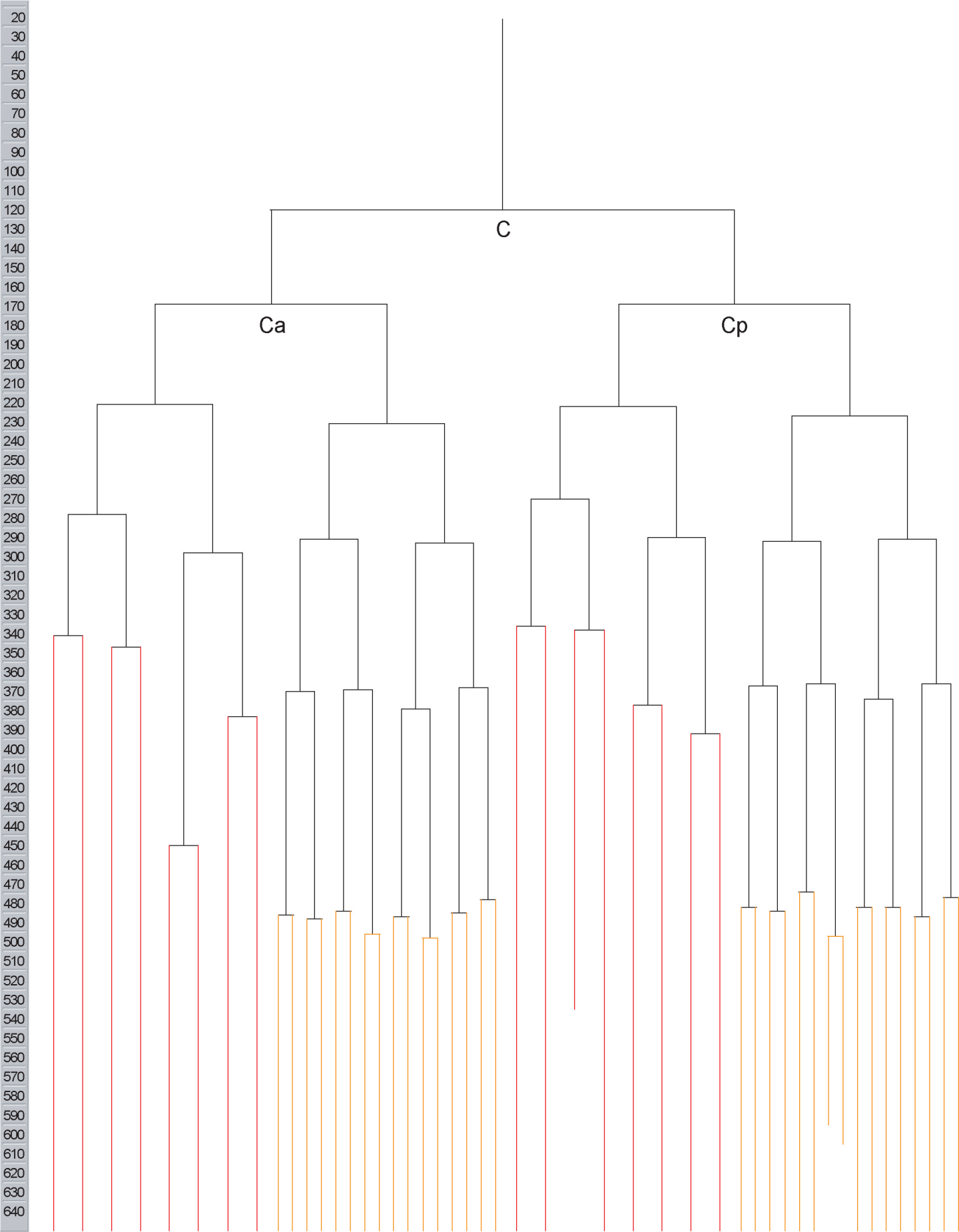
Epl
Epr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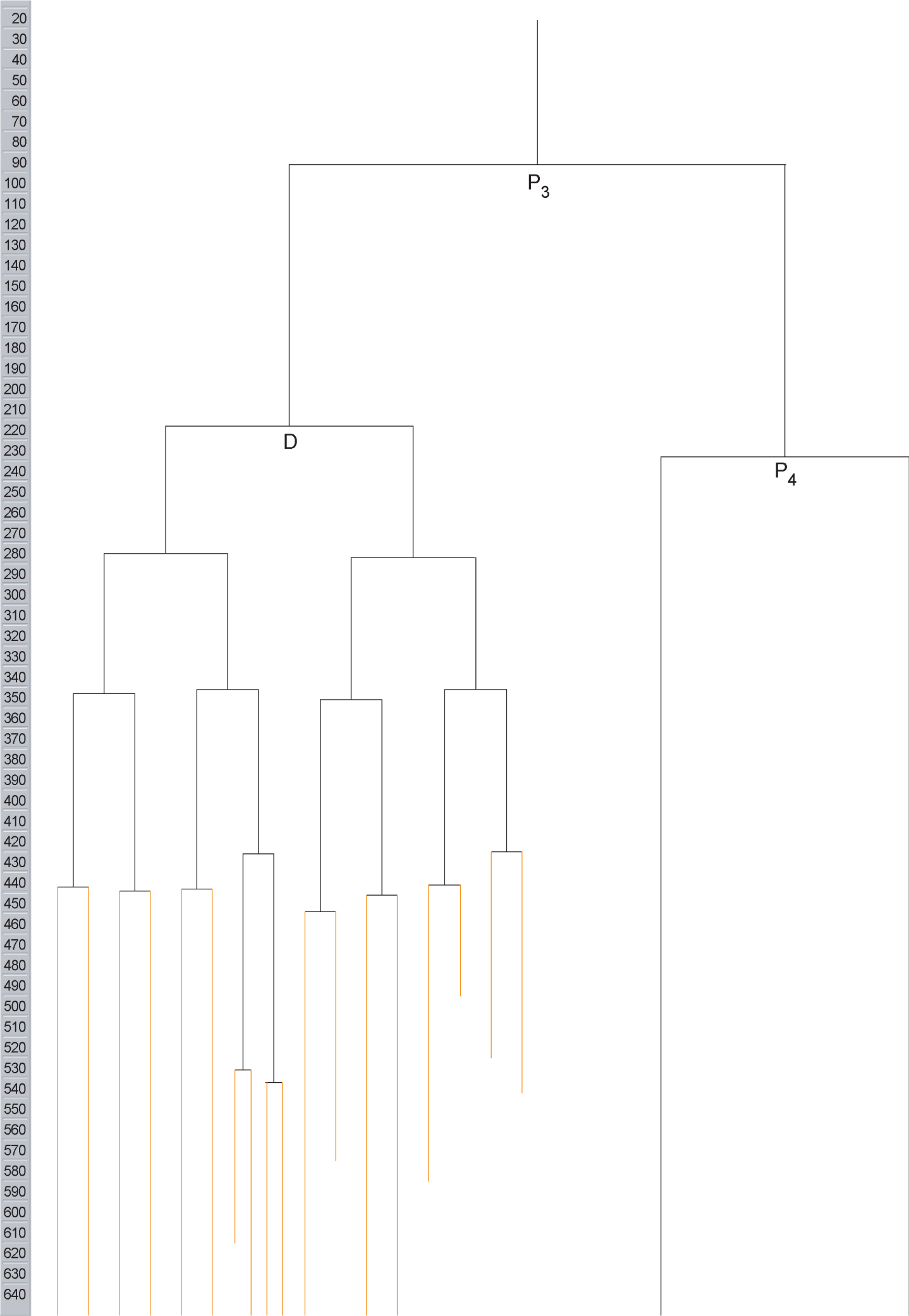
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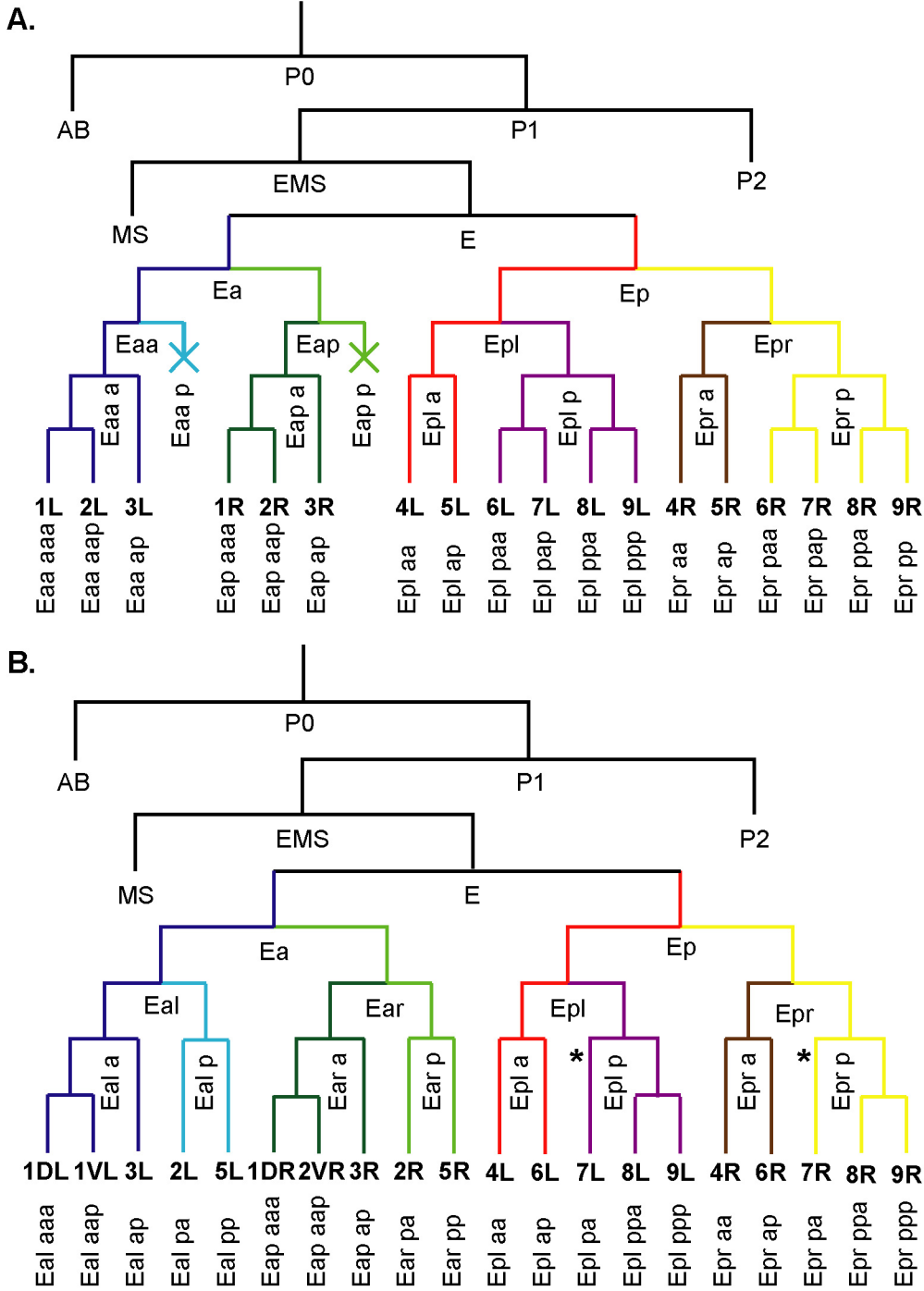
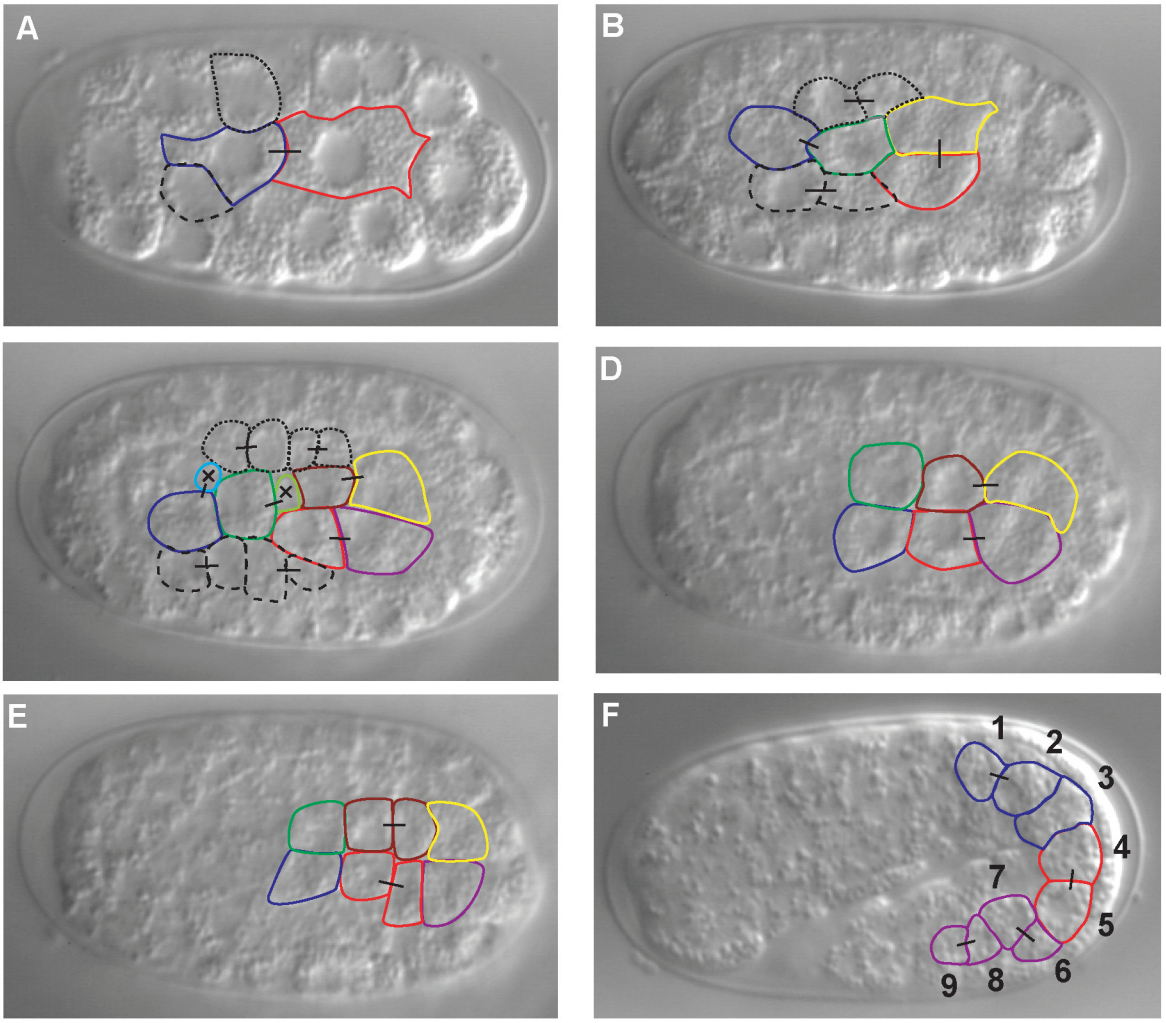
P4


A.
P0
AB
P1
EMS
P2
MS
E
Ea
Ep
Eaa
Eap
Epl
Epr
Eaa a
Eaa p
Eap a
Eap p
Epl a
Epl p
Epr a
Epr p
1L 2L 3L
1R 2R 3R
4L 5L 6L 7L 8L 9L
4R 5R 6R 7R 8R 9R
Eaa aaa
Eaa aap
Eaa ap
Eap aaa
Eap aap
Eap ap
Epl aa
Epl ap
Epl paa
Epl pap
Epl ppa
Epl ppp
Epr aa
Epr ap
Epr paa
Epr pap
Epr ppa
Epr pp
B.
P0
AB
P1
EMS
P2
MS
E
Ea
Ep
Eal
Ear
Epl
Epr
Eal a
Eal p
Ear a
Ear p
Epl a
Epl p
Epr a
Epr p
*
*
1DL 1VL 3L 2L 5L 1DR 2VR 3R 2R 5R 4L 6L 7L 8L 9L 4R 6R 7R 8R 9R
Eal aaa
Eal aap
Eal ap
Eal pa
Eal pp
Eap aaa
Eap aap
Eap ap
Ear pa
Ear pp
Epl aa
Epl ap
Epl pa
Epl ppa
Epl ppp
Epr aa
Epr ap
Epr pa
Epr ppa
Epr ppp


A
B
D
E
F
1
2
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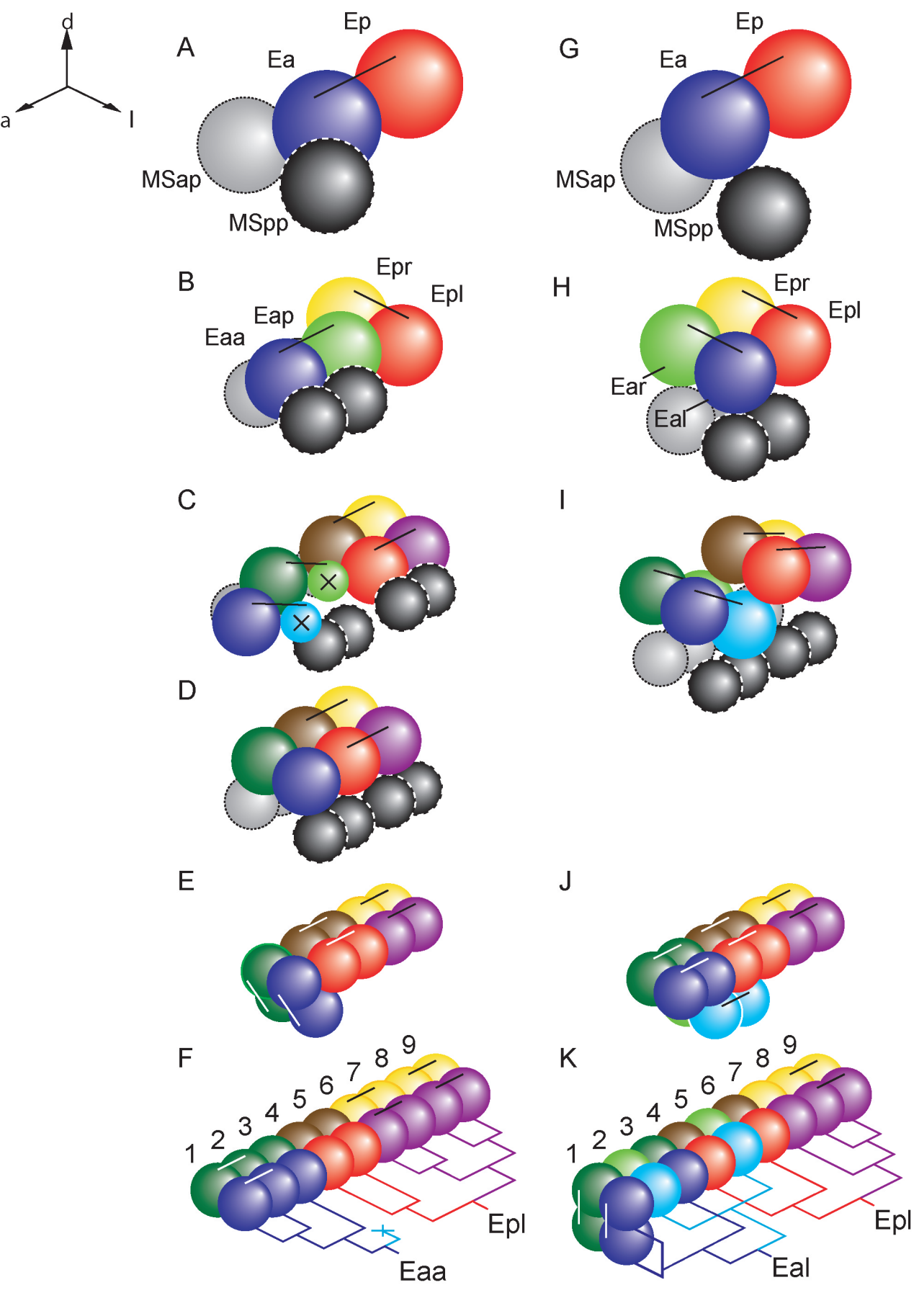
4
5
6
7
8
9


d
a
l
A
Ep
Ea
MSap
MSpp
B
Epr
Epl
Eap
Eaa
C
D
E
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1
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6
7
8
9
Epl
Eaa
G
Ep
Ea
MSap
MSpp
H
Epr
Epl
Ear
Eal
I
J
K
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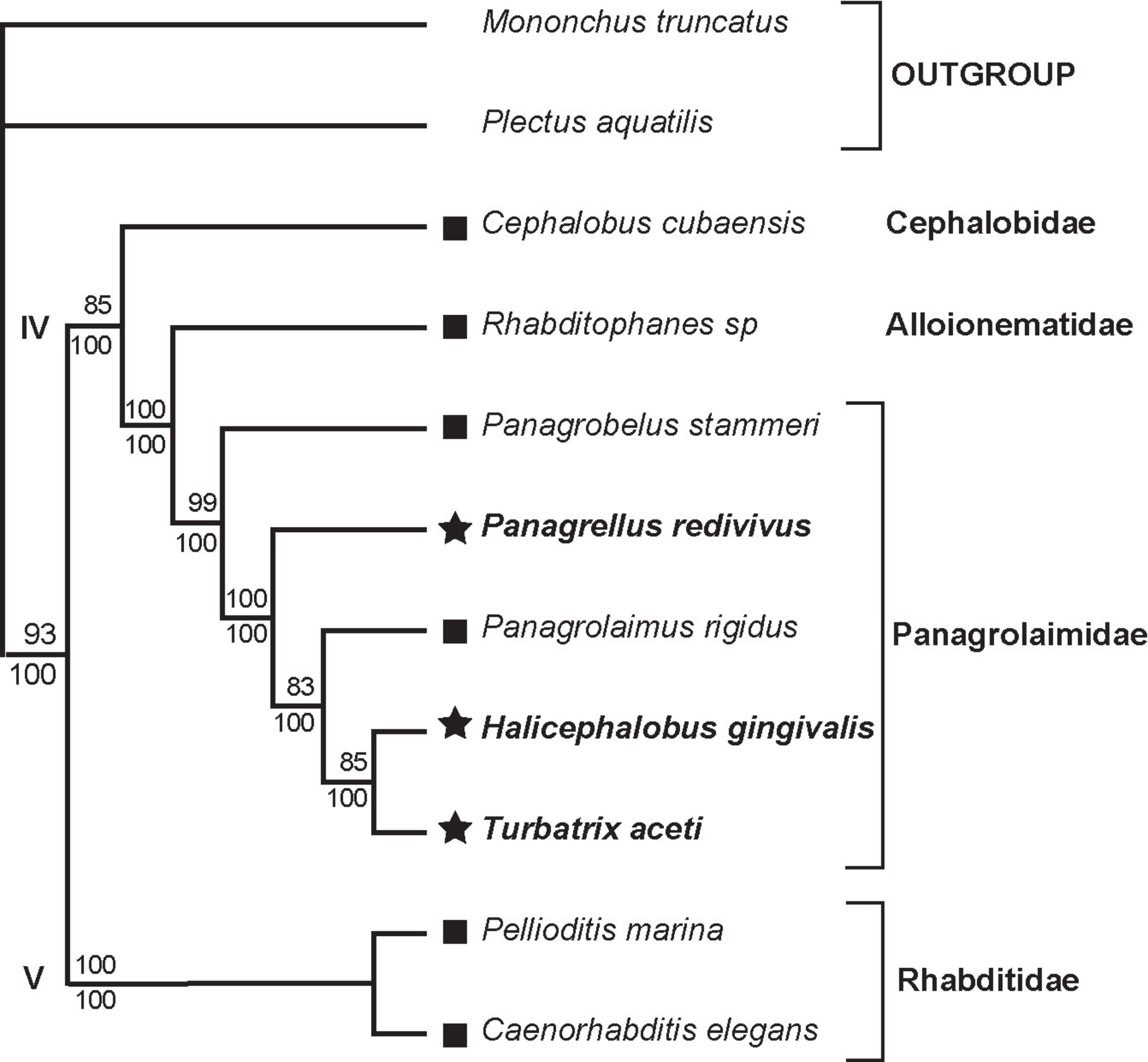
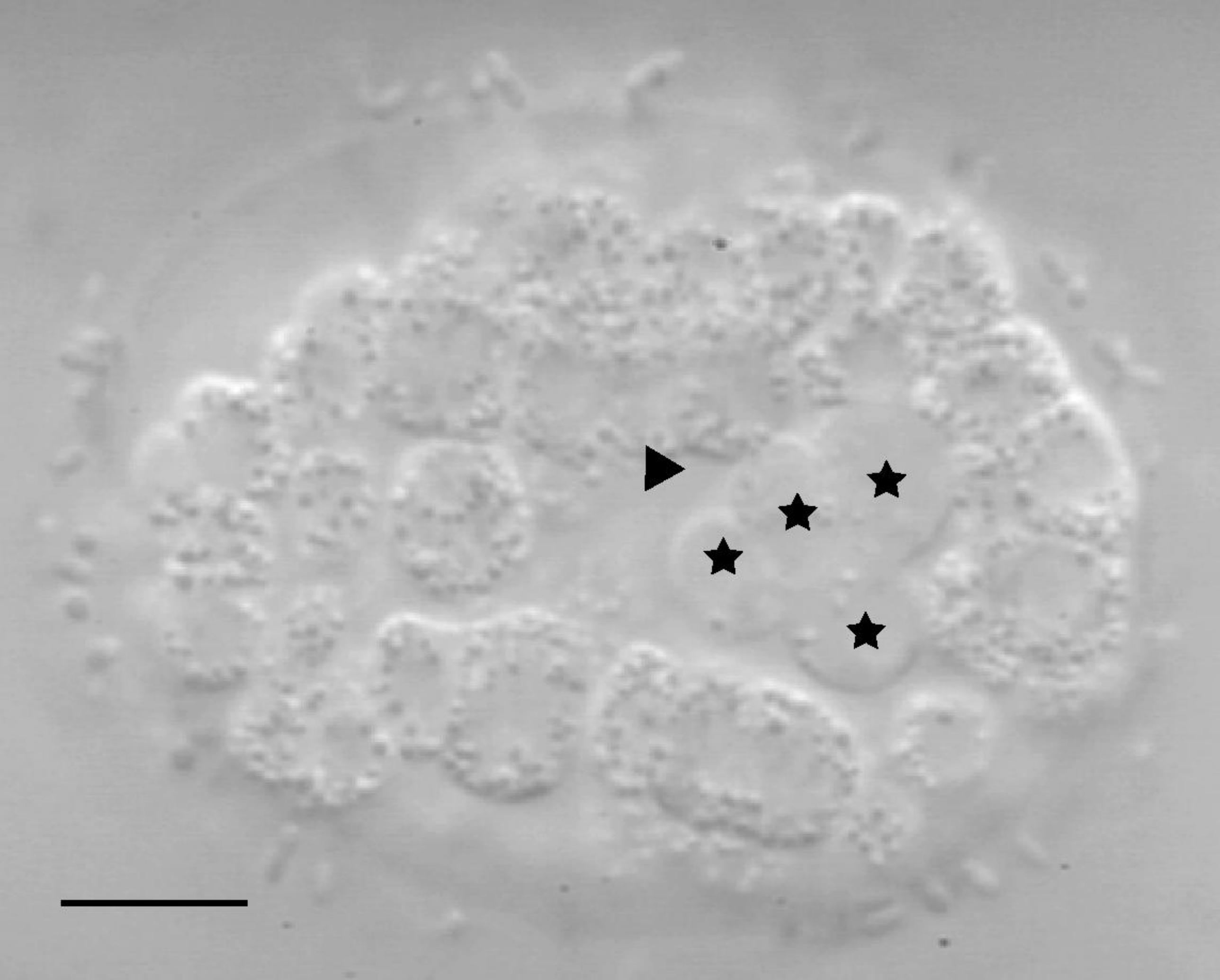
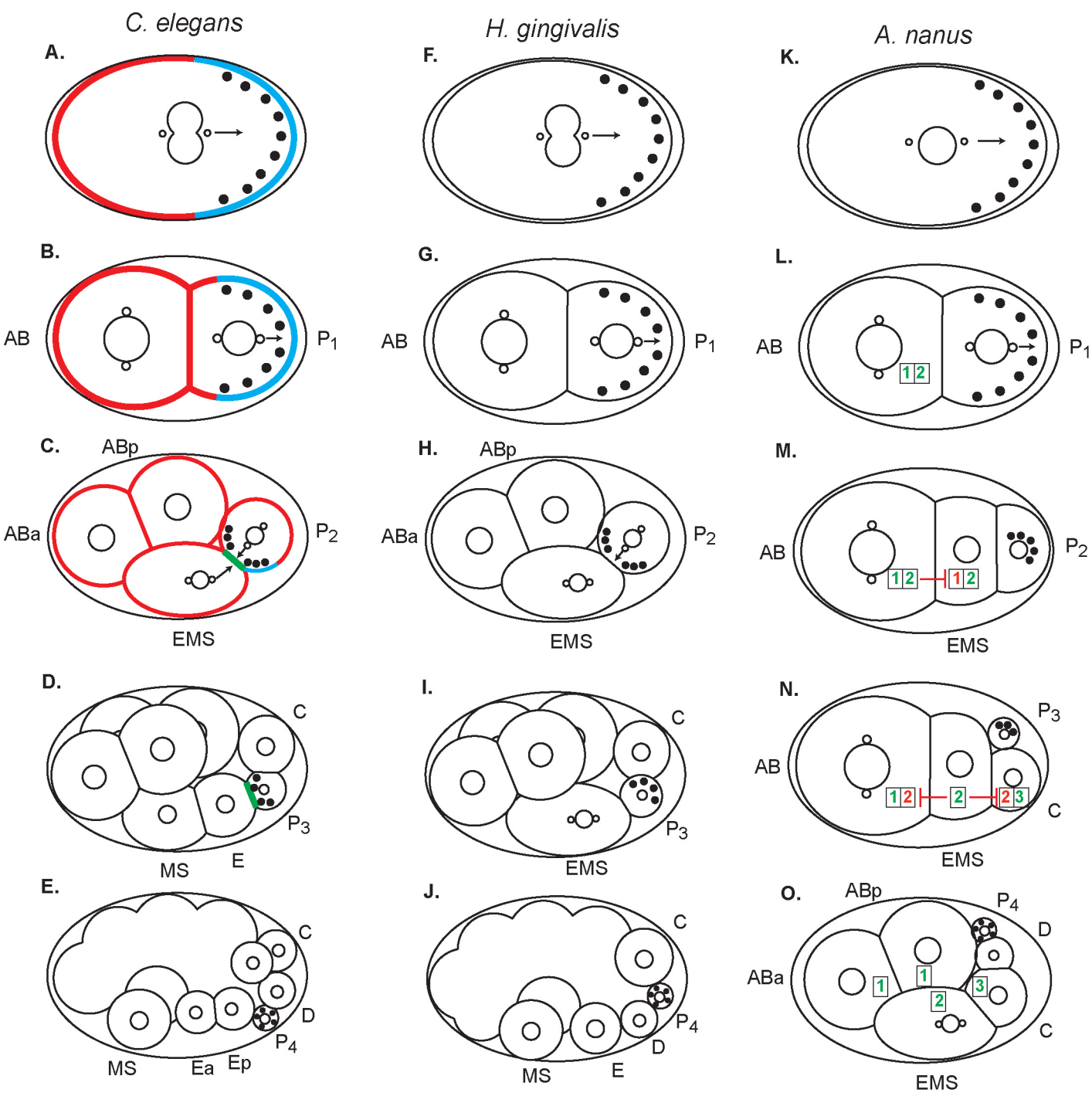
Epl
Eal


Mononchus truncatus
Plectus aquatilis
OUTGROUP
Cephalobus cubaensis
Cephalobidae
Rhabditophanes sp
Alloionematidae
Panagrobelus stammeri
Panagrellus redivivus
Panagrolaimus rigidus
Halicephalobus gingivalis
Turbatrix aceti
Panagrolaimidae
Pelliditis marina
Caenorhabditis elegans
Rhabditidae
IV
V
85
100
100
100
99
100
100
100
83
100
85
100
93
100
100
100




C. elegans
H. gingivalis
A. nanus
A.
F.
K.
B.
G.
L.
AB
P1
C.
H.
M.
ABp
ABa
P2
EMS
D.
I.
N.
C
P3
MS
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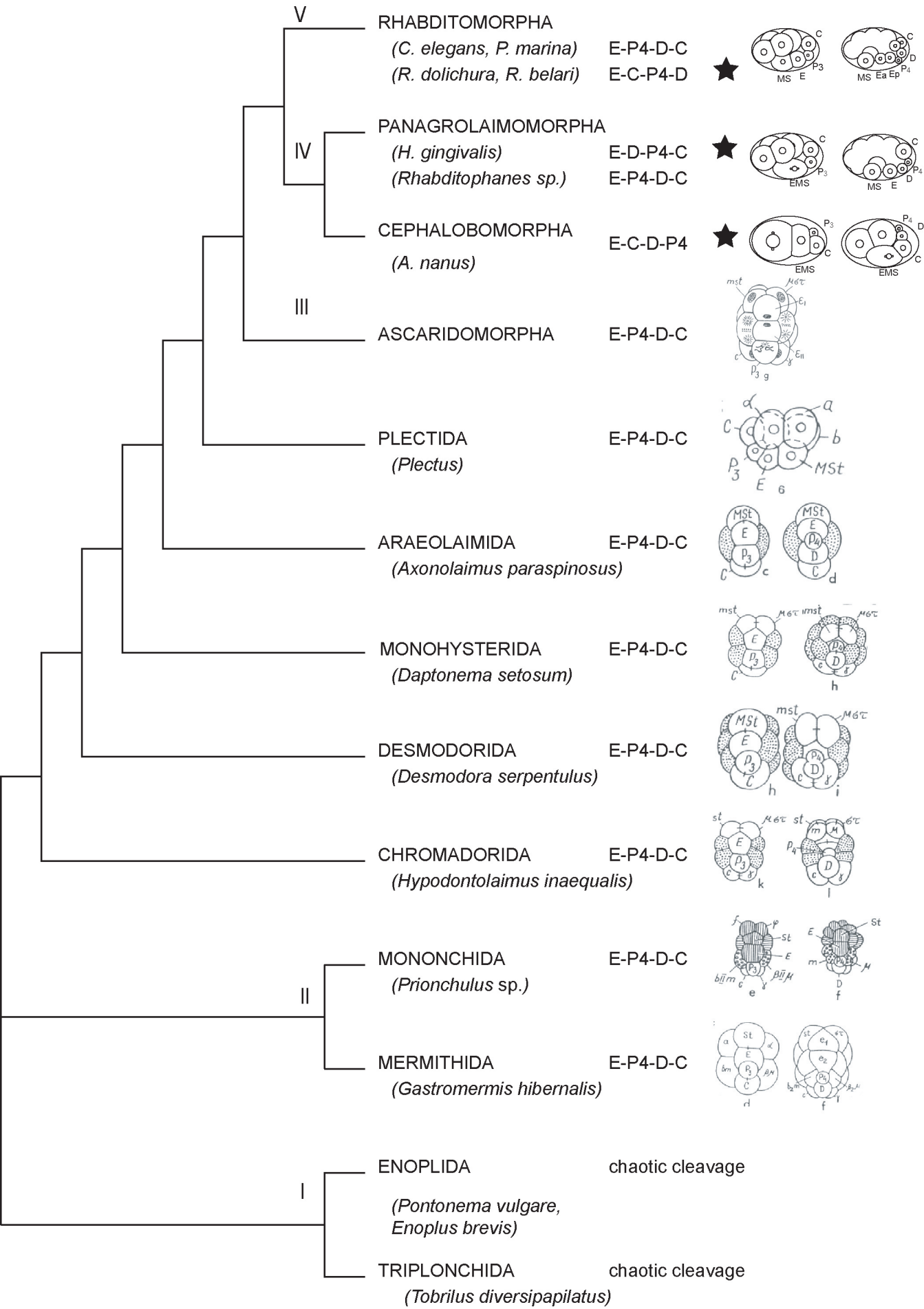
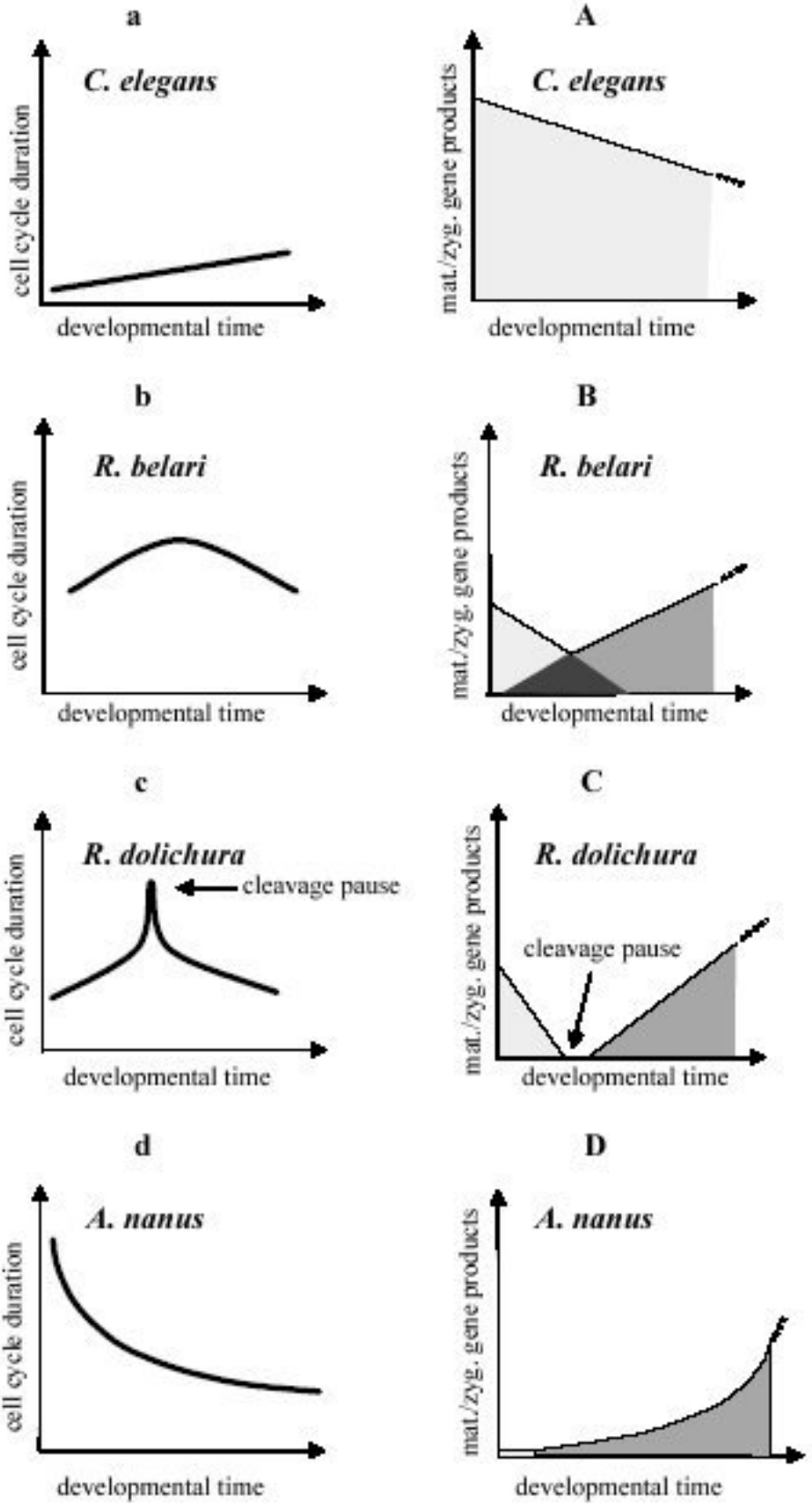
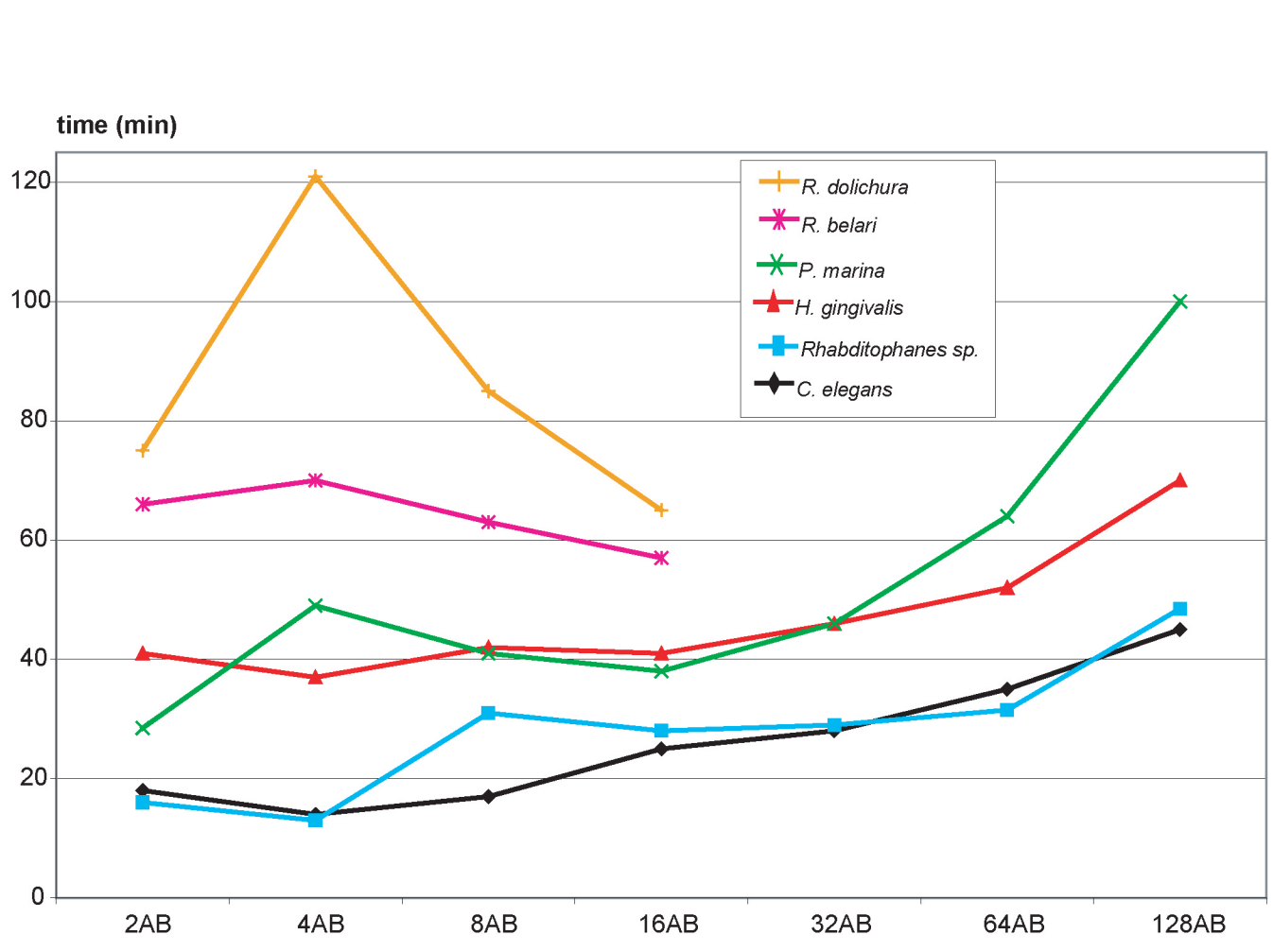
P4
D
Ea
Ep


V
RHABDITOMORPHA
(C. elegans, P. marina) E-P4-D-C
(R. dolichura, R. belari) E-C-P4-D
IV
PANAGROLAIMOMORPHA
(H. gingivalis) E-D-P4-C
(Rhabditophanes sp.) E-P4-D-C
CEPHALOBOMORPHA
E-C-D-P4
(A. nanus)
III
ASCARIDOMORPHA E-P4-D-C
PLECTIDA E-P4-D-C
(Plectus)
ARAEOLAIMIDA E-P4-D-C
(Axonolaimus paraspinosus)
MONOHYSTERIDA E-P4-D-C
(Daptonema setosum)
DESMODORIDA E-P4-D-C
(Desmodora serpentulus)
CHROMADORIDA E-P4-D-C
(Hypodontolaimus inaequalis)
II
MONONCHIDA E-P4-D-C
(Prionchulus sp.)
MERMITHIDA E-P4-D-C
(Gastromermis hibernalis)
I
ENOPLIDA chaotic cleavage
(Pontonema vulgare,
Enoplus brevis)
TRIPLONCHIDA chaotic cleavage
(Tobrilus diversipapilatus)

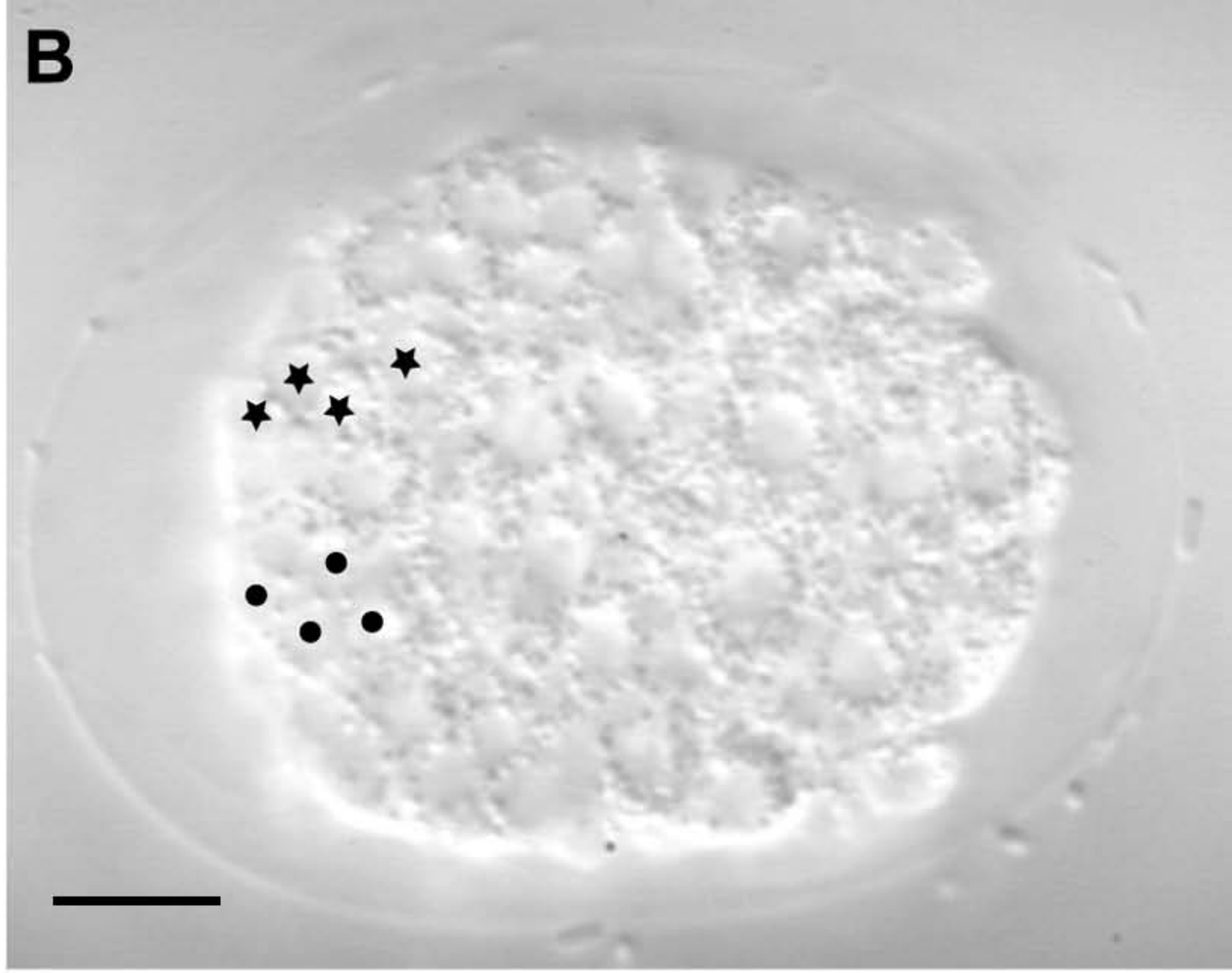
Observations (a-d):
Changes of cell cycle lengths in the AB lineage of early nematode embryos.

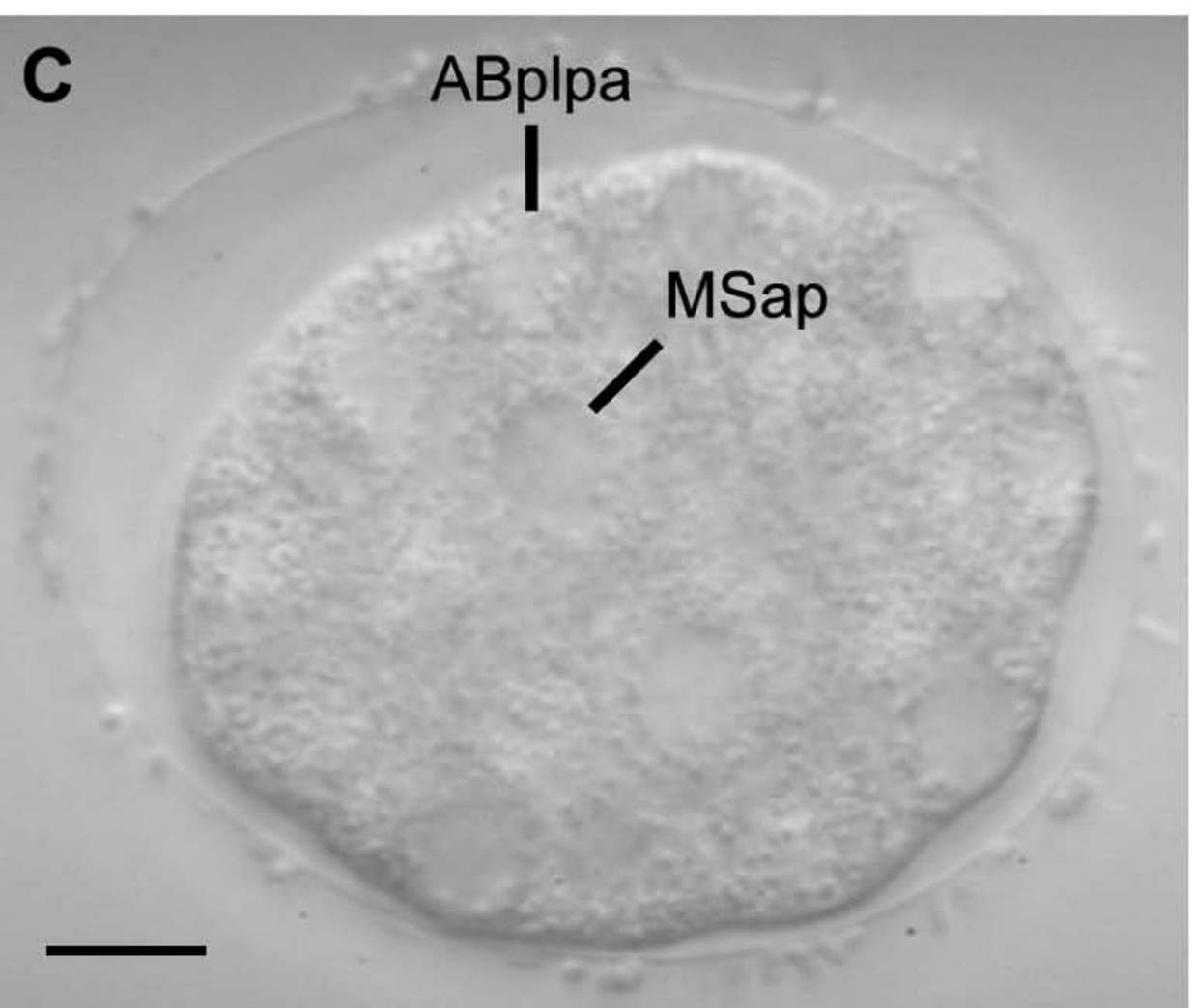
Interpretation (A-D):
Assumed availability of maternal and zygotic gene products relevant for cell cycle progression.

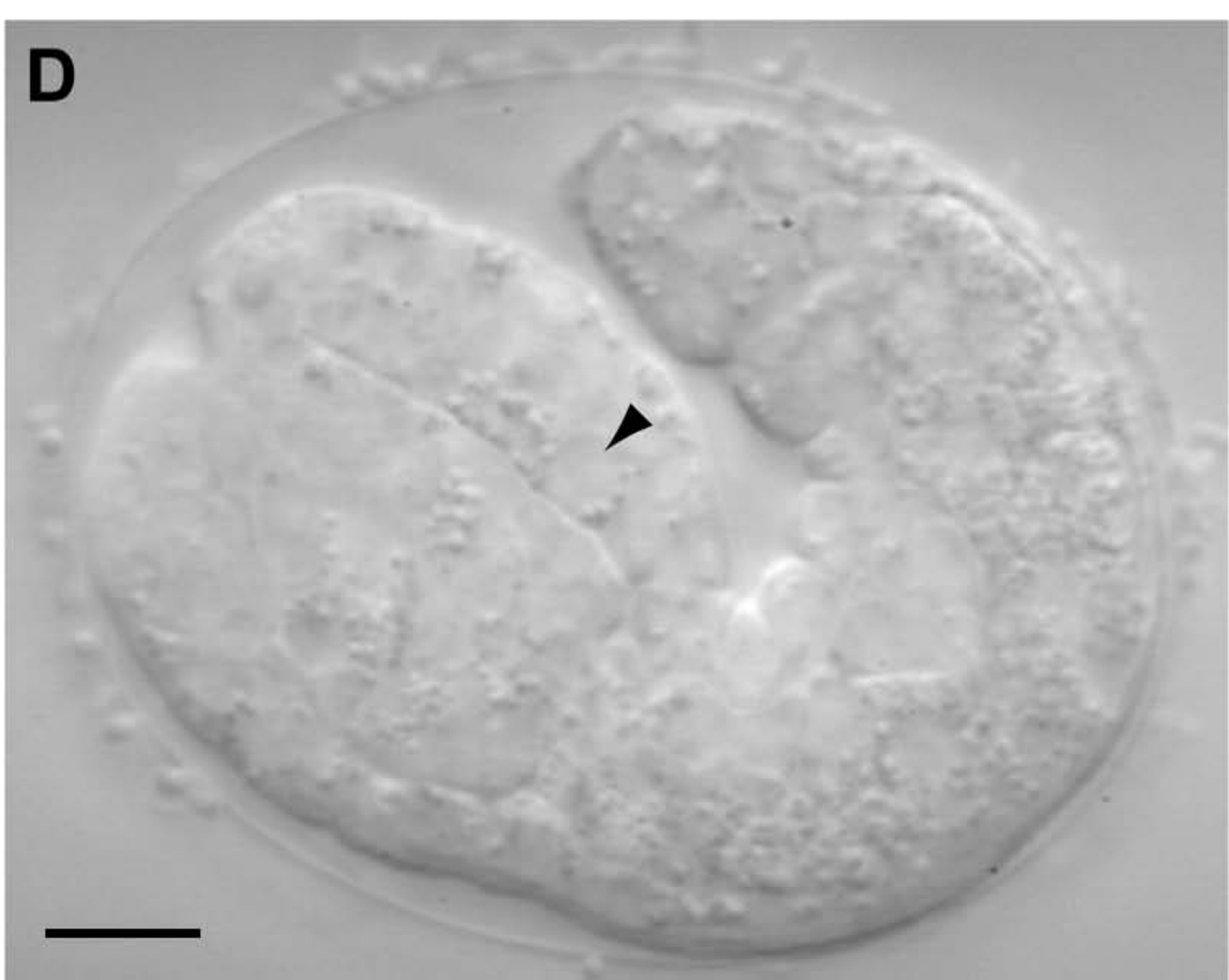



time (min)
120
100
80
60
40
20
0
2AB
4AB
8AB
16AB
32AB
64AB
128AB
R. dolichura
R. belari
P. marina
H. gingivalis
Rhabditophanes sp.
C. elegans


A
ABalap
ABplaa


B


C
ABplpa
MSap


D

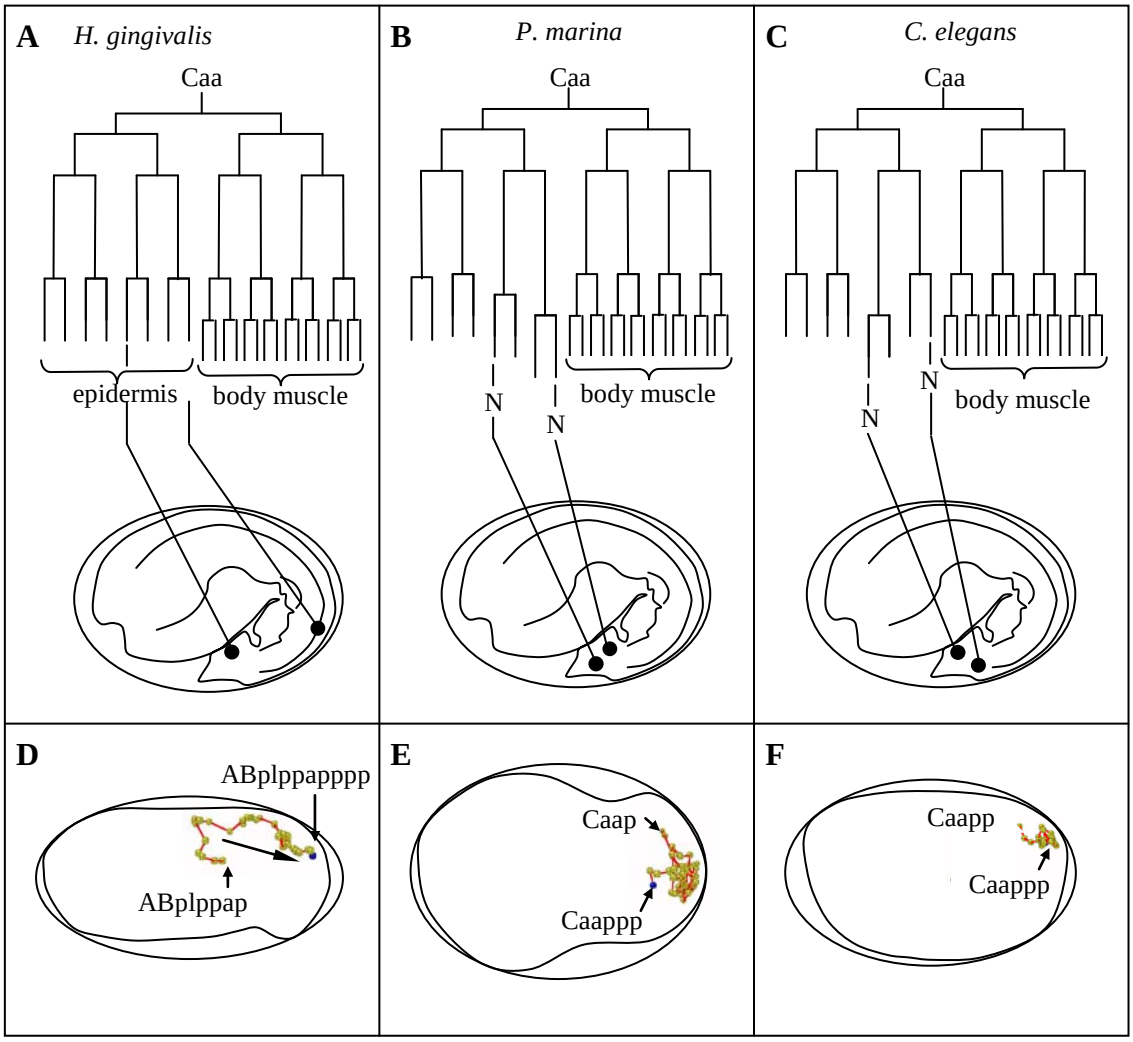

A
H. gingivalis
Caa
epidermis
body muscle
B
P. marina
Caa
N
N
body muscle
C
C. elegans
Caa
N
N
body muscle
D
ABplppapppp
ABplppap
E
Caap
Caappp
F
Caapp
Caappp

Table 1

	AB				MS				E				C				D				P4				total			
	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce
Pharynx	63	60	79	56	32	31	33	30																	95	91	112	86
Neuron	214	199	186	211	2	1	7	6					2		2	2									218	200	195	219
Muscle	1	1	1	1	29	33	28	28					32	32	32	32	20	20	20	20					82	86	81	81
Epidermis	72	74	114	66			3						14	16	14	13									86	90	131	79
Intestine									20	18	20	20													20	18	20	20
Other	16	18	20	20	10	7	10	11																	26	25	30	31
Gonad																					4	2	2	2	4	2	2	2
Mitosis				26				3																				29
Total survivors	366	352	400	380	73	72	81	78	20	18	20	20	48	48	48	47	20	20	20	20	4	2	2	2	531	512	571	547
Cell death	12	18	58	78	9	4	9	13		2															21	24	67	91
Unresolved mitosis	15	13			3																				18	13		
Unresolved cell	33	38			3	10																			36	48		
Total cells produced	426	421	458	458	88	86	90	91	20	20	20	20	48	48	48	47	20	20	20	20	4	2	2	2	606	597	638	638

Supplementary Table 2. Comparison of number of cells per terminal tissue type (row) of the 8 great granddaughters (column) between *Rhabditophanes* sp. (*Rh*), *H. gingivalis* (*Hg*), C*. elegans* (*Ce*) and *P. marina* (*Pm*).

	ala				alp				ara				arp				pla				plp				pra				prp			
	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce	Rh	Hg	Pm	Ce
Pharynx			3		22	18	24	17	40	42	40	39									1		11								1	
Neuron	54	37	46	42	29	28	19	24	11	14	18	13	7	4	8	12	12	8	12	13	36	40	21	41	21	27	23	23	44	41	39	43
Body muscle																													1	1	1	1
Epidermis			1		3	3	8	3	2		1	2	27	25	28	22	22	25	28	21	1	2	16	2	15	16	23	14	2	3	9	2
Other																					9	12	10	11				1	7	6	10	8
Total	54	37	50	42	54	49	51	44	53	56	59	54	34	29	36	34	34	33	40	34	47	54	58	54	36	43	46	38	54	51	60	54