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# Study of chromosome morphology of venerid clam *Paphia malabarica*

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## ABSTRACT

Among the exploited bivalve resources of India, clams are by far the most widely distributed and abundant. Good clam fishery is reported from the Ratnagiri coast of Maharashtra, India. It is represented mainly by shortneck clams, *Paphia malabarica*. Present study done from February 2013 to September 2014, analysed the morphological characters and the karyotypes of *P. malabarica*. The chromosome formula derived from the present investigations for this clam was 10m + 5sm + 4t (2n = 38). The karyotype displayed a high number of metacentric chromosomes. The percentage of euchromatin observed in both the long and short arms of the chromosomes of the *P. malabarica* was more compared to the heterochromatin, indicating active regions of replication and transcription.

**Key words:** *Paphia malabarica*, karyotypes, Ratnagiri, Maharashtra, India

## 1. INTRODUCTION

Subclass Heterodonta of the marine bivalve molluscs includes family Veneridae which has over 400 living species such as the edible clams, the cockles etc. This family includes 24 genus distributed worldwide. Among them, clams are the most prominent members belonging to genus *Paphia, Meretrix* and *Katelysia*. These clams support an artisanal fishery along the major estuaries i.e. Kalabadevi and Kajali estuaries along Ratnagiri coast. The shortneck clam *Paphia malabarica*, locally know as "Tisrya" is exploited throughout the year along this coast. They occur generally in estuaries and bays and in great demand due to their taste.

*P. malabarica is* a marine species. Both the front and hind margins were narrowly rounded and the ventral margin tends to have a very slight indentation towards the hind end. The surface of the shell was sculptured with strong, close-set, concentric ridges, which were raised and rounded, and not flattened. The separating interstitial grooves were also much deeper. As the concentric ridges and grooves were strictly parallel to the margin of shell, they were slightly flexed posteriorly in conformity with the slight indentation of the ventral margin towards the hind end. The hinge was having three, short, thick cardinal teeth. The tooth in front of the cardinals in the left valve and the hollow in the right are rudimentary. The pallial sinus was very deep and u-shaped. The inner surface was quite smooth throughout and its margin not denticulated. The lunule was relatively shorter and broad. The shell was of a pale yellowish brown colour,

indistinctly rayed with grayish brown bands. Sometimes the surface was more elaborately mottled with brownish angular markings all over.

Though the morphological characteristics based on the shell structure, shape, depth, concentric lines, ridges, and the cardinal teeth which can be used as taxonomical tools in the identification of these bivalves, variations in the colour patterns and markings are observed. Under stressful or unfavourable conditions, the shell morphology tends to change and may pose a problem in identifying the species. Chromosomes, being species specific, can prove an appropriate tool for the identification and comparison of the genetic relationship among the genus belonging to one family and species belonging to one genus (Gonza´lez-Tizo´n et. al., 2012).

This biological investigation and understanding the chromosome variations would help in correlating the genetic characteristics of this clam.

## 2. MATERIALS AND METHODS

Samples of *P. malbarica* (Shortneck clam) were collected monthly by using a drag net by hand picking. They were cleaned by a wire brush to remove adhered sand, mud and other organisms. Then they were placed in tubs filled with filtered sea water for 24 hrs. for acclimatization. The clams that had open valves after the acclimatization period were discarded. The healthy clams were used for the tissue treatment.

The clams were then kept in colchicine solution (0.005 %) for 6–8 hrs to arrest the chromosomes in Metaphase. Gills and gonads were separated from each treated clam dissected and treated twice with 0.56% KCl solution for 15 min at room temperature. For fixing they were treated by freshly prepared Carnoy's solution (ethanol: glacial acetic acid, 1:3 by volume) kept at 2- $4^0$ C. The solution was be changed 3-4 times every 15 min and then kept at $4^0$ C till chromosome preparation (Martínez et al., 2002; Ferna´ndez-Tajes et. al., 2003). About 1 $mm^2$ of gill tissue was cut and put in 0.25 ml of 60% acetic acid for dissociation. The tissue was then carefully ground with glass rod. Then 2 ml of 60 % acetic acid was added and kept for 5-10 min. The cell suspension was centrifuged at 1000 rpm for 2 min and the supernatant was then dropped from 30 – 100 cm height on to a clean glass slide preheated at 40 – $45^0$ C. The slides were then air dried and stained in 10% Giemsa in pH 6.8 phosphate buffer for 20 – 30 min. The slides were washed with distilled water and dried at room temperature.

For karyotyping, photographs of metaphase chromosomes spreads were taken with the help of a Leica DM 500 microscope and enlarged pictures were obtained. Individual chromosomes were cut out from the spread. Karyotypes were arranged by decreasing size of chromosomes and classified according to the centromeric index, following the nomenclature of Levan, et al., (1964). The chromosomes are then match up using size and centromere position as guides. Vernier calipers were used for measuring the length. Mean value of the length of the chromosome arms and the mean value for their total chromosome lengths were calculated for each of the chromosome pairs. Following calculations would be done from the observations.

$$\text{The relative length} = \frac{\text{Chromosome length}}{\text{Total length haploid set}} \times 100$$

$$\text{Centromeric index} = \frac{\text{Length of shorter arm of a chromosome}}{\text{Total chromosome length}} \times 100$$

$$\text{Arm ratio} = \frac{\text{Length of the long arm of the chromosome}}{\text{Length of the short arm}}$$

Chromosome measurements were also made using the computer application MicroMeasure version 3.3 (Reeves and Tear, 1999).

## 3. RESULT

The Karyomorphometric analysis of somatic metaphase complement of *Paphia malabarica* (Plate 1 to 3, Table 1) showed that the species had 10 metacentric (Nos. 1 to 7, 9, 14 and 15), 5 submetacentric (Nos.8 and 10 to 13) and 4 telocentric (Nos. 16 to 19) pairs of chromosomes (2n = 38). The Absolute length of the chromosomes ranged from 0.7 to 1.6. The Relative length ranged from 3.22 to



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7.37. The Centromeric index ranged from 0 to 50. The Arm ratio showed a variation between 1 to ∞. Fundamental arm number for *P. malabarica* was 64. The ideogram of the karyotype is represented by Fig. 1.

Measurements of the chromosomes were taken by using vernier calipers as well as by the MicroMeasure 3.3 software, during the current study. The marked chromosomes are represented by Plates 1 to 4. Point-like features along several chromatids have been marked: centromeres are marked with large circles and the segments are marked by green lines (Plate 4). The excel data generated by the software is represented in Table 2. The data generated by the software showed the length measurements of long and short arms, the total length of the chromosomes, arm ration and Centromeric index. The amount of euchromatin and heterochromatin in each arm were also calculated by the software MicroMeasure 3.3.

**Plate 1.** External features of shorneck clam, *P. malbarica*

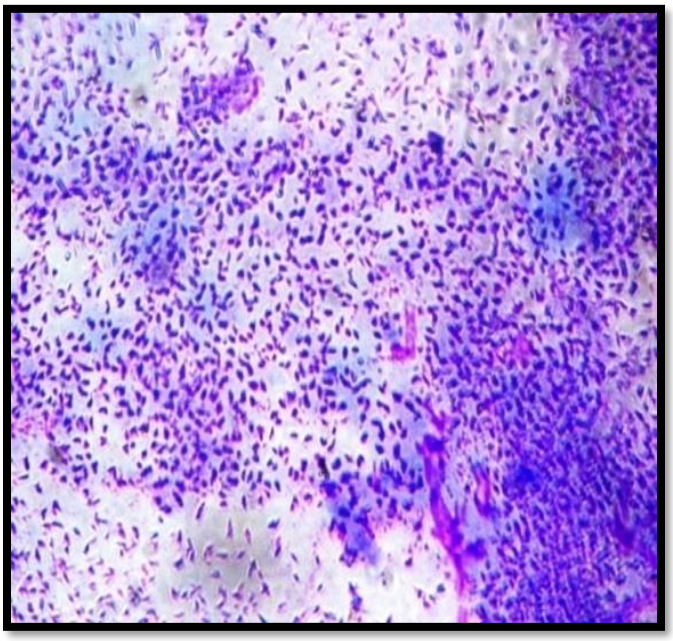

**Plate.2.** Giemsa-stained metaphase chromosomes of *P. malabarica*



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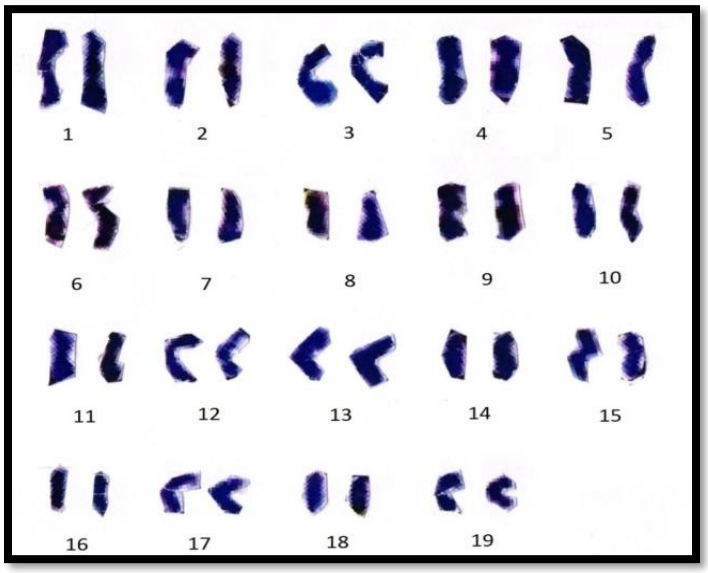


**Plate.3.** Karyotype of *P. malabarica*

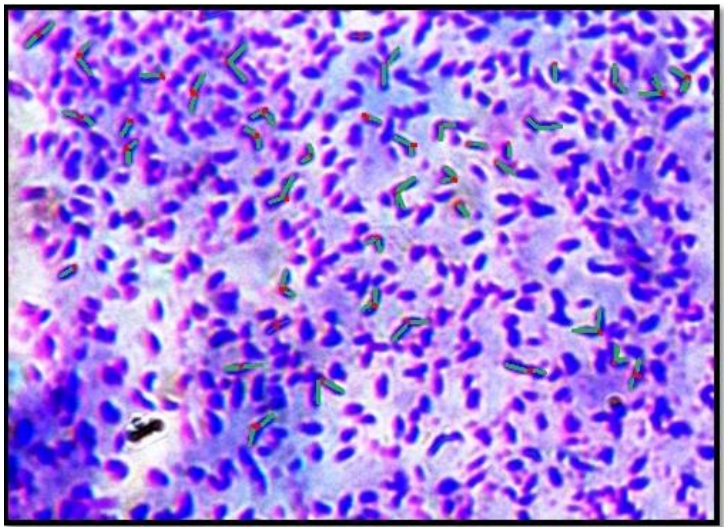

**Plate 4.** Chromosome measurements done by MicroMeasure 3.3 for *P. malabarica*

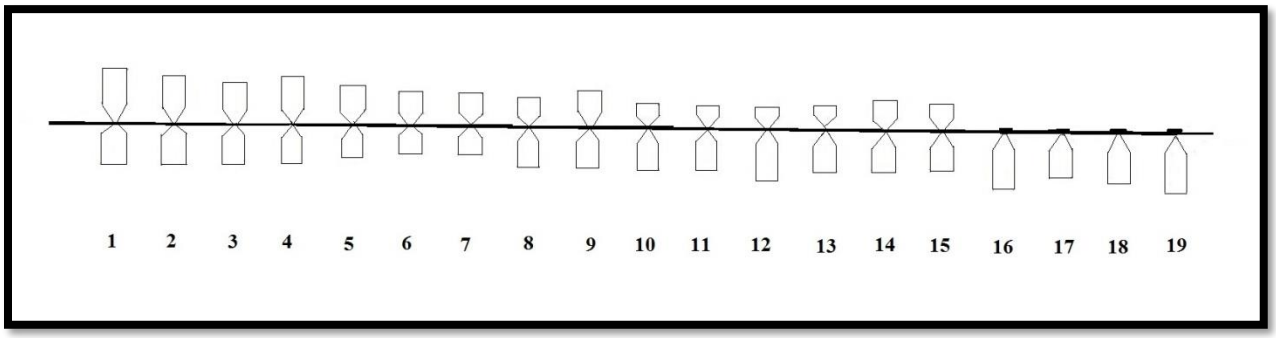


**Fig. 1.** Ideogram of *P. malabarica*



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**Table 1.** Karyomorphometric analysis of somatic metaphase complement of *Paphia malabarica*

| Chromosome pair No. | Length of long arm (L) (μm) | Length of short of arm (S) (μm) | Absolute length of chromosome (L+S) (μm) | Centromeric index=Short arm/Total length*100 | Arm ratio=Long arm/Short arm (L / S) | Relative length=Chromosome length/Total length haploid set*100 | Chromosome class |
|---|---|---|---|---|---|---|---|
| 1 | 0.8 | 0.8 | 1.6 | 50.0 | 1.0 | 7.37 | m |
| 2 | 0.8 | 0.7 | 1.5 | 46.67 | 1.14 | 6.91 | m |
| 3 | 0.7 | 0.6 | 1.3 | 46.15 | 1.17 | 5.99 | m |
| 4 | 0.7 | 0.7 | 1.4 | 50.0 | 1.0 | 6.45 | m |
| 5 | 0.6 | 0.6 | 1.2 | 50.0 | 1.0 | 5.52 | m |
| 6 | 0.6 | 0.6 | 1.2 | 50.0 | 1.0 | 5.52 | m |
| 7 | 0.5 | 0.5 | 1.0 | 50.0 | 1.0 | 4.60 | m |
| 8 | 0.7 | 0.4 | 1.1 | 36.37 | 1.75 | 5.06 | sm |
| 9 | 0.6 | 0.6 | 1.2 | 50.0 | 1.0 | 5.52 | m |
| 10 | 0.7 | 0.4 | 1.1 | 36.37 | 1.75 | 5.06 | sm |
| 11 | 0.9 | 0.5 | 1.4 | 35.71 | 1.8 | 6.45 | sm |
| 12 | 0.7 | 0.4 | 1.1 | 36.37 | 1.75 | 5.06 | sm |
| 13 | 0.7 | 0.4 | 1.1 | 36.37 | 1.75 | 5.06 | sm |
| 14 | 0.6 | 0.5 | 1.1 | 45.46 | 1.2 | 5.06 | m |
| 15 | 0.6 | 0.5 | 1.1 | 45.46 | 1.2 | 5.06 | m |
| 16 | 0.9 | - | 0.9 | 00 | ∞ | 4.14 | t |
| 17 | 0.7 | - | 0.7 | 00 | ∞ | 3.22 | t |
| 18 | 0.8 | - | 0.8 | 00 | ∞ | 3.68 | t |
| 19 | 0.9 | - | 0.9 | 00 | ∞ | 4.14 | t |
| Total | 13.1 | 8.2 | 21.7 | 664.93 | 18.51 | 88.83 | |

**Diploid chromosomes number = 38**
**Chromosomes formula = 10m + 5sm + 4t**
**Fundamental arm number = 64**
**Total complement length = 21.7**



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| Set ID: PM | |
|---|---|
| Project: | *P. malabarica* |
| Mag: | 600 |
| Image resolution:37.79 pixels per cm | |

**Table.2.** Excel data generated by MicroMeasure 3.3 software for *P. malabarica*

| Marking order | Rank | Length each | Long arm | Short arm | Arm Ratio (L/S) | Cent. Index (S/(L+S)) | Eu. in long arm | Het. in long arm | Eu. in short arm | Het. in short arm |
|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 1 | 16.65 | 8.87 | 7.78 | 1.14 | 0.47 | 8.87 | 0.000 | 7.77 | 0.00 |
| 6 | 2 | 13.45 | 7.36 | 6.09 | 1.21 | 0.45 | 7.36 | 0.000 | 6.08 | 0.00 |
| 15 | 3 | 12.94 | 7.98 | 4.96 | 1.61 | 0.38 | 7.38 | 0.00 | 4.96 | 0.00 |
| 4 | 4 | 10.95 | 6.37 | 4.58 | 1.39 | 0.42 | 6.37 | 0.00 | 4.58 | 0.00 |
| 14 | 5 | 10.90 | 5.70 | 5.20 | 1.09 | 0.48 | 5.70 | 0.00 | 5.20 | 0.00 |
| 8 | 6 | 10.51 | 7.18 | 3.32 | 2.16 | 0.32 | 0.00 | 7.18 | 3.32 | 0.00 |
| 12 | 7 | 10.18 | 5.24 | 4.94 | 1.06 | 0.48 | 5.24 | 0.00 | 4.94 | 0.00 |
| 16 | 8 | 10.16 | 6.38 | 3.77 | 1.69 | 0.37 | 6.38 | 0.00 | 0.00 | 3.76 |
| 10 | 9 | 9.09 | 4.60 | 4.49 | 1.02 | 0.49 | 4.60 | 0.00 | 4.49 | 0.00 |
| 5 | 10 | 9.01 | 5.23 | 3.76 | 1.39 | 0.42 | 5.23 | 0.00 | 3.77 | 0.00 |
| 17 | 11 | 8.94 | 4.74 | 4.19 | 1.13 | 0.46 | 0.00 | 4.74 | 0.00 | 4.19 |
| 1 | 12 | 8.93 | 4.55 | 4.37 | 1.04 | 0.49 | 4.55 | 0.00 | 4.37 | 0.00 |
| 7 | 13 | 8.90 | 4.79 | 4.10 | 1.16 | 0.46 | 0.00 | 4.79 | 4.11 | 0.00 |
| 18 | 14 | 8.88 | 5.39 | 3.48 | 1.54 | 0.39 | 8.87 | 5.39 | 0.00 | 3.48 |
| 3 | 15 | 8.69 | 4.77 | 3.91 | 1.21 | 0.45 | 0.00 | 0.00 | 4.77 | 0.00 |
| 19 | 16 | 5.08 | 5.08 | 0.00 | ∞ | 0.00 | 8.61 | 5.08 | 0.00 | 0.00 |
| 2 | 17 | 4.32 | 4.32 | 0.00 | ∞ | 0.00 | 8.35 | 4.32 | 0.00 | 0.00 |
| 11 | 18 | 4.19 | 4.19 | 0.00 | ∞ | 0.00 | 8.21 | 4.19 | 0.00 | 0.00 |
| 13 | 19 | 3.90 | 3.90 | 0.00 | ∞ | 0.00 | 7.77 | 3.90 | 0.0 | 0.00 |

## 4. DISCUSSION

In recent years, the number of cytogenetic studies on bivalves has remarkably increased. Since 1992, karyotypes of about 65 species have been studied by banding techniques (Thiriot-Quievreux, 2002). Lin et. al (2008) reported that chromosome spreads of bivalves were usually prepared with three methods using different tissues: gonad, embryos, and adult gills.

They got more mitotic metaphase plates from adult gill chromosome preparation in *M. mercenaria*, than other species, such as *M. meretrix* (Lu et al., 2003) and *Tegillarca granosa* (Zheng et al., 1996), and better chromosomal morphologies. During the present study, chromosome spreads were prepared by using gonad and adult gill tissues of the selected clams. Better results were obtained from the adult gill tissues. The chromosome number reported in the shortneck clam, *P. malabarica* during the present study *was* 2n = 38. Within the bivalve class this is the most frequent chromosome number (Thiriot-Quievreux, 2002). Their karyotypes are very different and could be used for identification of one species from another. This also suggests the rearrangement of the chromosomes during the divergence of the species. The chromosome formula reported for the species is 10m + 5sm + 4t (2n = 38). Based on double arm (m, sm) and single arm (st, t) chromosomes, double arm chromosomes were the majority in this species. Chromosomal forms in



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the subgenus according to Surget-Groba et al., (2001), karyotypes with high number of metacentric or submetacentric chromosomes are considered as more apomorphic than those with a high number of telocentric chromosomes.

The MicroMeasure software employed in the present study, allowed marking the chromosomes and related features had been carried out, by using length measurements including total length, arm length, arm ratio, and centromere index and exported to Microsoft Excel spreadsheets. The chromosome segments or the chromatids as well as the centromeres were marked and lengths were measured by the software. During the current study, the percentage of euchromatin observed in both the long and short arms of the chromosomes of the clam species was more compared to the heterochromatin, indicating active regions of replication and transcription. This also indicated that the phenotypic expression of genes and also the variations observed in the clam species were due to the euchromatin. The morphological and structural variations within the species could be well attributed to the amount of euchromatin present in the chromatids. No satellite chromosomes were observed during the present investigations.

Ming-y et. al., (2012) studied the karyotype of *P. undulate* and reported that the length and the arm ratio of chromosomes were measured and calculated with Micromeasure 3.2 software. The results showed that the chromosome number of *P.undulate* was 38 and the karyotype formula was summarized as 2n = 38. The formula derived was 14m+12sm+4st+8t.The number of chromosome arm was 64. No satellite chromosome had been found in the chromosome set of *P.undulate*. The length of chromosome was about 1.5-4.0 μm.

Karyotyping studies have been done for different species belonging to the families, Nuculidae, Arcidae, Pectinidae, Unionidae, Cardiidae, Mactridae, Tellinidae, Psammobiidae, Corbiculidae, Veneridae (Thiriot-Quievreux, 2002) and Donacidae (Martı´nez et al., 2002). Several workers have observed the chromosomes of bivalves (pelecypod molluscs) using squash or air-drying techniques (Ahmed and Sparks, 1967, 1970; Longwell, et. al., 1967; Menzel, 1968; Patterson, 1970; Ieyama and Inaba, 1974; Ieyama, 1977). The chromosome numbers and karyotypes of about 157 species of bivalve molluscs were reported by Zhang et al., (2000) , and the chromosome numbers of 66 species ( 42% ) of them were 2n = 38. Thirty eight chromosomes were the most frequently found among the bivalve molluscs. Chromosome numbers of Veneridae were reported as of three types, i.e, 2n = 28, 30, 38 (Zhang et al., 2000; Que et al., 1999; Borsa and Thiriot-Quiévreux, 1990). The most common type is 2 n = 38.Wang et al., (1999) believed that 2 n = 38 seem to be the initial diploid type of bivalve mollusc.

The chromosomal changes accompanying bivalve evolution are an area about which few reports have been published. Pérez-García et. al., (2014) reported about the chromosomes of five species of venerid clams (*Venerupis corrugata, Ruditapes philippinarum, Ruditapes decussatus, Dosinia exoleta,* and *Venus verrucosa*). Both the chromosome numbers and the karyotypes determined in this work for *Ruditapes philippinarum, R. decussatus, Venerupis corrugata, Venus verrucosa,* and *Dosinia exoleta* were in agreement with previous results (Borsa and Thiriot-Quiévreux, 1990; Insua and Thiriot-Quiévreux, 1992; Ebied & Aly, 2004 and Hurtado & Pasantes, 2005) and further confirm that, unlike other families within the order Veneroida in which interspecific differences in chromosome numbers have been detected, all Veneridae species have the same chromosome number, 2n = 38.

During the evolution of animals, the smaller telocentric chromosomes were the initial forms, while the bigger metacentric chromosomes were the derived forms (Ojima, 1983). It appears that in the course of evolution, the karyotype of Veneridae derived a major number of chromosomes in the latter form, which seems to be consistent with some morphological features of this family.

## 5. CONCLUSION

Family: Veneridae is the most rich and diverse family of heterodont bivalves with high ecological and economic value. *Paphia malbarica* (Shortneck clam) is one of the commercially important clams belonging to this family and it is exploited extensively along the Ratnagiri coast of Maharashtra. The shell morphology is the taxonomic tool used currently for the identification of this clam. But under stressful or unfavourable conditions, the shell morphology tends to change and may pose a problem in identifying the species, especially from the export point of view. The karyotyping of *P. malabarica* would help in correctly identifying the species and also to understand the genetic characteristics of this clam species.

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**Declaration of conflicting interests**

The authors declare that there are no conflicts of interests.

**Data and materials availability**



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All data associated with this study are present in the paper.

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