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Shubham Rao
Department of Entomology, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chhattisgarh, India

Shashidhar Viraktamath
Department of Entomology, University of Agricultural Sciences, GKVK, Bengaluru, India

SS Shaw
Department of Entomology, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chhattisgarh, India

Corresponding Author:
Shubham Rao
Department of Entomology, Indira Gandhi Krishi Vishwa Vidyalaya, Raipur, Chhattisgarh, India

Morphometry of stingless bees of the genus *Tetragonula* from three biogeographical regions of Chhattisgarh

Shubham Rao, Shashidhar Viraktamath and SS Shaw

Abstract
Stingless bees from three biogeographical regions of Chhattisgarh (Bastar plateau, Chhattisgarh plains and North hills) were collected and their morphometry was studied by selecting 37 morphological parameters to understand the diversity. The data were subjected to Principal Component analysis and Canonical Discriminant analysis. Bees from Chhattisgarh plains were bigger measuring 3.54 mm in length and 1.52 mm mm in head width compared to Bastar plateau (3.50 and 1.49 mm) and North hills (3.53 and 1.49 mm). Similarly, forewings were longer (3.61 mm) and wider (1.29 mm) in the bees from Chhattisgarh plains. Both Principal Component and Canonical Discriminant analysis resulted in overlapping but widely distributed clusters indicating greater variation among the bees of these three regions and existence of more than one species in Chhattisgarh. Further studies are needed to identify the species based on male bees and their genitalia.

Keywords: Stingless bees, morphometry, *Tetragonula*, biogeographical regions, Chhattisgarh

1. Introduction
Stingless bees are the smallest bees which belong to the class Hexapoda, order Hymenoptera, Family Apidae and tribe Meliponini. They date back on the earth about 65 million years ago and are found in tropics and subtropics (Baumagartner and Roubik 1989) [3]. These are eusocial, non Apis bees and corbiculate in nature. These bees are unique with reduced wing venation, presence of the penicillium, rigid setae on the outer apical margin of the hind tibia (Wille, 1983) [1]. Stingless bee, name itself implies that they have reduced sting and lack venom apparatus but possess bidentate mandibles with which they try to bite to defend the colony. They also try to irritate by entering nose, ears, eyes and stick to hairs when colony is disturbed. *Oxytrigona* bees secrete a mandibular secretion that cause a painful blister. The honey produced by these bees is of lesser quantity hence these bees are less used commercially. Due to their flexible behavior, smaller size, stingless bees can adopt to most of regions and easy to culture. Natural colonies of stingless bees are perennial in nature, building their nest in dark enclosures like cavities in old hollow trees and their branches, anthills, termitaria, crevices and cracks, old mud walls with cracks, electric boards, rock cracks, iron pipes, pots, in ground and other hidden places which are not easily recognized. Only the hive entrance is visible outside and whole colony is concealed inside the crevices. There are about 14 species of stingless bees occurring in India that belong to three genera namely *Tetragonula*, *Lepidotrigona* and *Lisotrigona*. Of these, the genus *Tetragonula* is the most dominant (Rasmussen, 2013; Viraktamath *et al*, 2020; Viraktamath and Shishira 2020) [4, 6, 7]. Chhattisgarh known as a bowl of rice has three agro-climatic zones namely Bastar plateau, Chhattisgarh plains and North hills of Chhattisgarh.
Bastar plateau and Chhattisgarh plains are known for rich biodiversity. The stingless bees are also the part of this rich diversity but are not so common subject of the study. In Chhattisgarh region, studies on foraging behavior of stingless bees have been made by Painkra and Mallaiah (2019) [5] and few more studies on the behavior of the stingless bees. However, our knowledge on diversity is very limited in this region. Hence, an effort was made to study the morphometry of stingless bees of the genus *Tetragonula* from three different regions of Chhattisgarh to understand the diversity of these bees as a first to identify the species. Results of morphometry of female bees are presented in this paper.

2. Materials and Methods
The samples of the stingless bees were collected from three different biogeographic regions of Chhattisgarh. Bastar Plateau included Kondagaon, Bastar, Dantewada, Sukma, Bijapur, Narayanpur. Chhattisgarh plains included Kanker, Gariyaband, Mungeli, Kawardha, and

Ambikapur. North hills of Chhattisgarh included Balrampur and Jashpur.

The method described by Viraktamath *et al.* (2020) [6] was adopted to collect the samples of bees. A water trap was set up just below the entrance of each colony of stingless bees in different places. Bees trapped in each colony were collected and stored in 95% ethyl alcohol vials separately and labeled. Each sample was examined under the stereoscopic binocular microscope and keyed out to the genus by using the key provided by Rasmussen (2013) [4] at the Insect Systematic laboratory, Department of Entomology, University of Agricultural Sciences, Gandhi Krishi Vignana Kendra, Bengaluru. The genus *Tetragonula* is characterized by the presence of longitudinal bands of hairs on the mesoscutum, sericeous area on the ventral surface of hind basitarsus and extension of scutellum beyond propodeum. Later the bees were sexed, counted and recorded separately. There were 8331 specimens amongst which 8306 were females and 25 males. Male bees were identified by the presence of male genitalial parts projecting from tip of abdomen. From each place, 20 bees were mounted on a card point, labeled and 10 bees were used for detailed morphometry studies. Remaining 10 bees were preserved dry as reference specimens.

Thirty-seven morphological parameters were selected to study the morphometry of the bees (Ruttner 1988; Rasmussen, 2013) [4]. The morphological characters included body length (BL),Head Width (HW), Head Length (HL), Eye Length (EL), Eye Width(EW), Inter Ocellar Distance(IOD), Diameter of Median Ocellus (DMO), Upper Inter Orbital Distance (UIOD), Ocello Orbital Distance (OOD), Length of Clypeus (CL), Maximum Width of Clypeus (CW), Length of Malar Space (MS), Length of Scape (SCL), Width of Scape (SCW), Length of Pedicel+Flagellomeres (FL), Length of first Flagellomere (1st FL), Length of second Flagellomere (2nd FL), Length of third Flagellomere (3rd FL), (Width of third Flagellomere (3rd Fw), Length of Mandible (MNL), Maximum Width of Mandible (MNW), Length of Forewing Including Tegula (FWL), Width of Forewing (FWW), Length of Pterostigma (PTL), Length of Marginal Cell (MCL), Maximum Width of Marginal Cell (MCW), Distance b/t M Cu bifurcation & basal tip of MC (DMCU), Number of Hamuli (HAM), Length of Mesoscutum (MSCL), Width of Mesoscutum (MSCW), Length of Scutellum (SCTL), Width of Scutellum(SCTW), Length of Hind Tibia (HTL), Width of Hind Tibia (HTW), Length of Hind Basitarsus (BTL), Width of Hind Basitarsus (BTW), Sericeous Area (SA). All the measurements were made under the stereoscopic binocular microscope fitted with the ocular micrometer at Bengaluru and expressed in millimeter except the hamuli which were counted. Data for females and males were pooled based on the biogeographical regions separately. Mean and standard deviation for each parameter were calculated. Data of the females were subjected to factor analysis which included analysis of variation, Principal Component analysis (PCA) and Canonical Discriminant analysis (CDA). However, as the samples of males were few the data was not analyzed further.

3. Results and Discussion

Detailed morphometry of each biogeographical region is presented in Table 1. The bees collected from Bastar Plateau had the body length of 3.5mm, head width 1.49 mm; fore wing length 3.56 mm; fore wing width 1.28 mm; hind tibial length 1.42 mm; hind tibial width 0.51 mm; hind basitarsus length 0.48 mm; hind basitarsus width 0.26 mm.

The bees collected from Chhattisgarh plains had the body length of 3.54 mm, head width 1.52 mm; Head length 1.17 mm; fore wing length 3.61 mm; fore wing width 1.29 mm; hind tibial length 1.43 mm; hind tibial width 0.51 mm; hind basitarsus length 0.49 mm; hind basitarsus width 0.26 mm.

The bees collected from North hills of Chhattisgarh had the body length of 3.53 mm, head width 1.49 mm; Head length 1.15 mm; fore wing length 3.59 mm; fore wing width 1.29 mm; hind tibial length 1.44 mm; hind tibial width 0.50mm; hind basitarsus length 0.48 mm; hind basitarsus width 0.26 mm.

The morphological variations were observed with respect to the geographic regions. The samples collected from Chhattisgarh plains had higher body length, head width, head length, eye length, mandible length when compared to the samples collected from Bastar Plateau and the samples of North hills of Chhattisgarh.

Principal Component analysis of morphometry data of 415 bees from three agroclimatic regions of Chhattisgarh resulted in 11 principal components with Eigen values more than 1.00 which explained the variation in agroclimatic zones to the extent of 59.10 per cent (Table 3). In the Principal Component 1, morphological parameters like, CL, CW, HL, HTL, SCTL, FL, MSCW, SCL, EL and MSCL had higher component loading that ranged from 0.406 to 0.710 and attributed to for 59.10 per cent variation. Principal Component 2 included parameters like BTW, HTW, MNL and IOD with component loading ranging from 0.307 to 0.705 which explained the variation to the extent of 7.08 per cent (Table 3). Principal Components 1 and 2 together explained the variation to the tune of 25.26 per cent.

Results of factor analysis and grouping of agroclimatic regions are presented in Figure 1 which revealed that there was no grouping of bees in distinct clusters indicating that there is lot of variation among the bees of these three agroclimatic regions of Chhattisgarh. It also indicated that more than one species may be occurring in these biogeographical regions as some of the bee samples were quite wide spread in the graph.

Canonical Discriminant analysis resulted in extraction of two functions with less than 1.0 Eigen values. Morphological parameters like HW, FWL, BTL, MNL, EL, FL and SCL had loading factors ranging from 0.21 to 0.363. In the second Canonical function, morphological parameters *viz.* DMCU, CW, OOD, IFL,SCTL and HAM had 0.229 to 0.403 loading (Table 4 & 5).

The scatter plot of Functions 1 and 2 (Figure 2) revealed overlapping clusters of all the three agroclimatic regions but the centroids of each biogeographical region were deviated away from each other. These results indicated great variation among the stingless bee population of these three agroclimatic regions which also indicated existence of more than one species as revealed in PCA.

Classification results and the percentage of accuracy of the grouping of the samples of different geographical regions of Chhattisgarh are presented in the Table 6. The results revealed that 69.2% of original grouped cases were correctly classified but in cross validation, only 63.1% of the grouped cases were found to be correct. These results corroborate the earlier results depicted by the PCA and CDA scatter plots. As many as 36.9% of the samples were not classified correctly which clearly pointed out the existence of greater variation in stingless bee population which in turn indicate occurrence of more than one species in Chhattisgarh. Hence it is concluded that further detailed studies are needed to identify the species in these biogeographical regions of Chhattisgarh.

Table 1: Morphometry of female bees of *Tetragonula* spp in three agroclimatic regions of Chhattisgarh

Sl. No.	Parameter	Morphometry of female bees in					
		Bastrar plateau		Chhattisgarh plains		North hills of Chhattisgarh	
		Mean (mm)	Standard deviation	Mean (mm)	Standard deviation	Mean (mm)	Standard deviation
1	BL	3.5	0.145	3.54	0.173	3.53	0.2
2	HW	1.49	0.06	1.52	0.06	1.49	0.05
3	HL	1.16	0.06	1.17	0.06	1.15	0.05
4	EL	1.01	0.04	1.03	0.05	1.01	0.04
5	EW	0.36	0.05	0.36	0.04	0.35	0.04
6	UIOD	1.00	0.03	1.00	0.04	0.99	0.02
7	DMO	0.15	0.00	0.15	0.00	0.15	0.00
8	IOD	0.40	0.03	0.39	0.03	0.39	0.03
9	OOD	0.20	0.02	0.20	0.01	0.21	0.02
10	CL	0.41	0.06	0.42	0.04	0.40	0.05
11	CW	0.57	0.05	0.56	0.04	0.58	0.04
12	LMS	0.06	0.05	0.05	0.01	0.06	0.01
13	SCL	0.54	0.03	0.55	0.03	0.54	0.02
14	SCW	0.10	0.00	0.10	0.00	0.10	0.00
15	FL	1.16	0.06	1.18	0.06	1.16	0.07
16	IFL	0.07	0.01	0.07	0.00	0.07	0.00
17	IIFL	0.10	0.00	0.10	0.00	0.10	0.00
18	IIIFL	0.10	0.00	0.10	0.00	0.10	0.00
19	IIIFW	0.12	0.00	0.12	0.00	0.13	0.00
20	MNL	0.58	0.04	0.59	0.03	0.58	0.03
21	MNW	0.23	0.03	0.23	0.06	0.24	0.03
22	FWL	3.56	0.12	3.61	0.14	3.59	0.11
23	FWW	1.28	0.06	1.29	0.06	1.29	0.05
24	PTL	0.50	0.02	0.50	0.02	0.50	0.01
25	MCL	1.18	0.05	1.17	0.06	1.18	0.04
26	MCW	0.26	0.02	0.26	0.02	0.26	0.02
27	DMCU	0.99	0.05	0.98	0.07	0.96	0.05
28	HAM	5.04	0.26	5.02	0.13	5.11	0.31
29	MSCL	0.94	0.05	0.95	0.04	0.93	0.04
30	MSCW	1.08	0.05	1.08	0.06	1.09	0. 05
31	SCTL	0.33	0.03	0.33	0.02	0.32	0.03
32	SCTW	0.70	0.06	0.71	0.42	0.71	0.04
33	HTL	1.42	0.08	1.43	0.08	1.44	0.05
34	HTW	0.51	0.02	0.51	0.02	0.50	0.02
35	BTL	0.48	0.02	0.49	0.03	0.48	0.04
36	BTW	0.26	0.02	0.26	0.02	0.26	0.02
37	SA	0.20	0.03	0.21	0.13	0.21	0.03

Table 2: Total variation in the morphometry of *Tetragonula* female bees of three agroclimatic regions

Total Variance Explained									
Component	Initial Eigenvalues			Extraction Sums of Squared Loadings			Rotation Sums of Squared Loadings		
	Total	% of Variance	Cumulative%	Total	% of Variance	Cumulative%	Total	% of Variance	Cumulative%
1	6.72	18.17	18.17	6.72	18.17	18.17	3.64	9.84	9.84
2	2.62	7.09	25.26	2.62	7.09	25.26	2.91	7.87	17.71
3	2.12	5.73	30.99	2.12	5.73	30.99	2.39	6.47	24.17
4	1.79	4.82	35.81	1.79	4.82	35.81	2.39	6.46	30.63
5	1.59	4.30	40.12	1.59	4.30	40.12	2.16	5.84	36.48
6	1.50	4.06	44.18	1.50	4.06	44.18	1.98	5.34	41.81
7	1.25	3.39	47.56	1.25	3.39	47.56	1.54	4.17	45.98
8	1.13	3.06	50.63	1.13	3.06	50.63	1.30	3.51	49.50
9	1.10	2.97	53.60	1.10	2.97	53.60	1.27	3.43	52.93
10	1.03	2.80	56.39	1.03	2.80	56.39	1.19	3.22	56.14
11	1.00	2.71	59.10	1.00	2.71	59.10	1.09	2.96	59.10
12	0.96	2.59	61.69						
13	0.92	2.48	64.17						
14	0.89	2.39	66.57						
15	0.85	2.29	68.86						
16	0.80	2.16	71.02						
17	0.77	2.08	73.10						
18	0.75	2.02	75.12						
19	0.72	1.94	77.06						
20	0.71	1.93	78.99						
21	0.70	1.90	80.89						
22	0.65	1.76	82.65						

23	0.62	1.69	84.33						
24	0.60	1.63	85.96						
25	0.59	1.59	87.55						
26	0.55	1.48	89.02						
27	0.51	1.37	90.40						
28	0.47	1.26	91.66						
29	0.45	1.21	92.87						
30	0.40	1.08	93.95						
31	0.37	1.00	94.95						
32	0.35	0.96	95.91						
33	0.35	0.94	96.84						
34	0.35	0.93	97.78						
35	0.30	0.82	98.60						
36	0.28	0.75	99.34						
37	0.24	0.66	100.00						
Extraction Method: Principal Component Analysis.									

Table 3: Rotated component matrix of morphometric parameters of *Tetragonula* female bees of three agroclimatic regions

Rotated Component Matrix[a]											
	Component										
	1	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
CL	0.71										
CW	0.67										
HL	0.57			0.33							
HTL	0.48	0.41									
SCTW	0.48						0.43				
FL	0.48	0.42									
MSCW	0.48	0.39									
SCL	0.48										
EL	0.43	0.31									
MSCL	0.41	0.40									
BTW		0.71									
HTW		0.64									
MNL		0.51									
IOD		0.31									
HW			0.70								
EW			0.69								
SCTL			0.58								
DMCU			0.50	0.43				-0.32			
IFL	0.33		-0.46		0.35						
BL	0.33		0.37					0.34			
FWW				0.68							
MCW				0.66							
MCL	0.31			0.61							
FWL	0.39	0.45		0.54							
IIIFL					-0.84						
BTL					0.79						
UIOD		0.32			0.38					0.35	
SCW						-0.85					
IIIFW						0.83					
IIFL						0.50				-0.37	
LMS							0.73				
HAM							0.67				
PTL								0.72			
DMO									-0.68		
MNW									0.68		
OOD					0.32					-0.69	
SA											0.84
Extraction Method: Principal Component Analysis. Rotation Method: Varimax with Kaiser Normalization. a. Rotation converged in 22 iterations.											

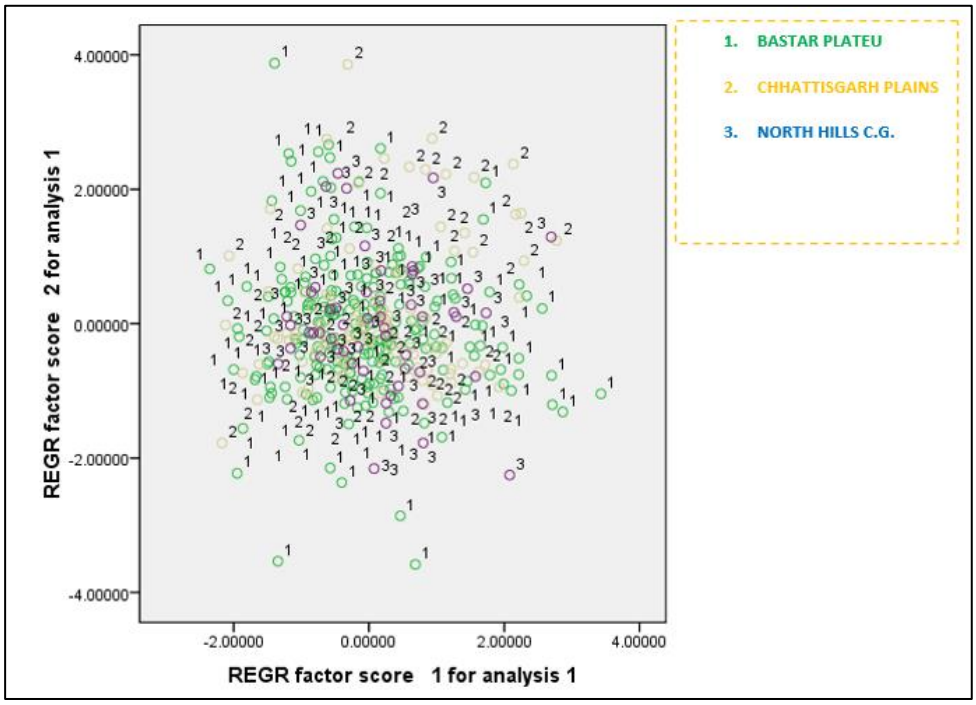

Fig 1: Factor analysis scatter plot showing clusters of *Tetragonula* female bees from three agroclimatic regions

Table 4: Canonical discriminant functions of *Tetragonula* female bees from three agro-climatic regions of Chhattisgarh

Eigenvalues				
Function	**Eigenvalue**	**% of Variance**	**Cumulative%**	**Canonical Correlation**
1	0.361[a]	61.9	61.9	0.515
2	0.223[a]	38.1	100.0	0.427
a. First 2 Canonical discriminant functions were used in the analysis.				

Table 5: Structure matrix of the morphometric parameters in Canonical discriminant functions

Structure Matrix		
Parameters	**Function 1**	**Function 2**
HW	-0.0363*	0.087
FWL	-0.241*	-0.112
BTL	-0.226*	0.005
MNL	-0.220*	0.081
EL	-0.219*	0.208
FL	-0.212*	0.088
SCL	-0.210*	-0.013
IOD	0.185*	0.123
HL	-0.182*	0.133
MSCL	-0.176*	0.120
FWW	-0.158*	-0.081
BL	-0.150*	-0.053
SA	-0.115*	-0.065
SCW	0.110*	0.028
MCL	0.104*	-0.019
LMS	0.099*	0.049
MSCW	0.092*	-0.089
DMCU	0.086	0.403*
CW	0.116	-0.282*
OOD	0.055	-0.247*
IFL	-0.183	-0.243*
SCTL	-0.116	0.239*
HAM	0.071	-0.229*
UIOD	-0.100	0.195*
HTL	-0.140	-0.142*
SCTW	-0.093	-0.133*
EW	-0.045	0.133*
IIIFL	0.110	0.127*
CL	-0.086	0.126*
HTW	-0.086	0.122*
MNW	0.044	-0.113*
BTW	-0.039	-0.107*

PTL	-0.050	-0.074*
MCW	0.031	0.063*
DMO	0.017	-0.055*
IIIFW	0.030	-0.045*
IIFL	-0.022	-0.036*
Pooled within-groups correlations between discriminating variables and standardized Canonical discriminant functions Variables ordered by absolute size of correlation within function.		
*. Largest absolute correlation between each variable and any discriminant function		

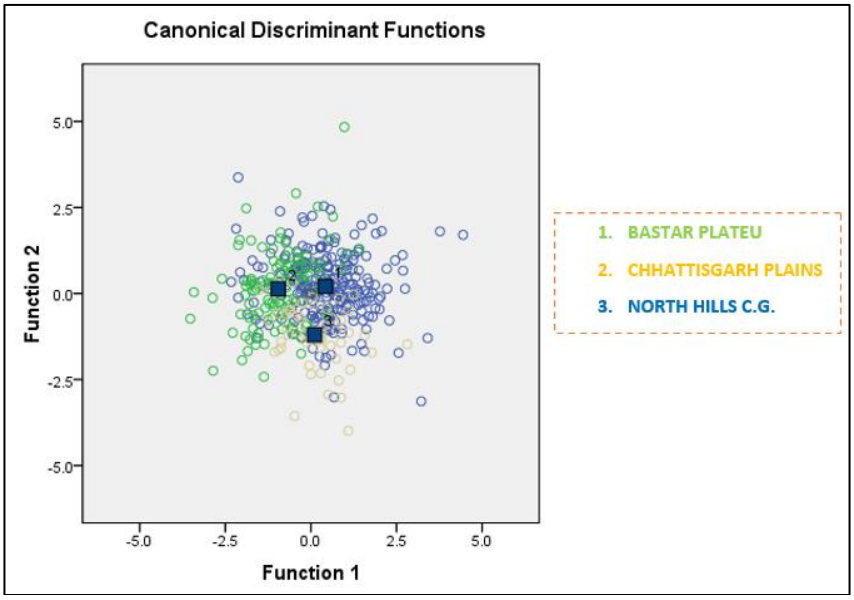

Fig 2: Canonical discriminant analysis scatter plot showing clusters of female bees from three agroclimatic regions of Chhattisgarh

Table 6: Classification results of *Tetragonula* female bees from three geographic regions of Chhattisgarh

Classification Results[b,c]						
		AGR Code	Predicted Group Membership			Total
			1	2	3	
Original	Count	1	212	26	7	245
		2	52	61	2	115
		3	35	6	14	55
		Ungrouped cases	1	0	0	1
	%	1	86.5	10.6	2.9	100.0
		2	45.2	53.0	1.7	100.0
		3	63.6	10.9	25.5	100.0
		Ungrouped cases	100.0	.0	.0	100.0
Cross-validated[a]	Count	1	201	33	11	245
		2	59	52	4	115
		3	36	10	9	55
	%	1	82.0	13.5	4.5	100.0
		2	51.3	45.2	3.5	100.0
		3	65.5	18.2	16.4	100.0
a. Cross validation is done only for those cases in the analysis. In cross validation, each case is classified by the functions derived from all cases other than that case.						
b. 69.2% of original grouped cases correctly classified.						
c. 63.1% of cross-validated grouped cases correctly classified.						

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