Supplementary Information

Table S1. Primers used for probe template (length of at least 600 bases) design. RNA Polymerase promoter sequences (T7 resp. T3) were incorporated in primer sequences.

Species	Opsin	Primer	Sequence
N. sammara	*SWS2*	SWS2_forward	5'-TAATACGACTCACTATAGGGATCACTCAGCCCGTTCTTGG-3'
		SWS2_reverse	5'-AATTAACCCTCACTAAAGGGCTCAGATCGAAGGTCTGCCC-3'
	RH2A-1	RH2A-1_forward	5'- TAATACGACTCACTATAGGGAAACCTTCGATGTGGACTGAAT-3'
		RH2A-1_reverse	5'-AATTAACCCTCACTAAAGGGGACACCTGTCAGGGCATTCC-3'
	RH2A-2	RH2A-2_forward	5'-TAATACGACTCACTATAGGGTAACCCTCGATGTGGACTGAGC -3'
		RH2A-2_reverse	5'-AATTAACCCTCACTAAAGGGTTCATCATGGCAAACTCCAACA-3'
M. berndti	*SWS2A*	SWS2A_forward	5'-TAATACGACTCACTATAGGGCTCAGGACCACTTGGGGAAC-3'
		SWS2A_reverse	5'-AATTAACCCTCACTAAAGGGGACTGGCTGATGACTCCTCG-3'
	RH2B	RH2B_forward	5'-TAATACGACTCACTATAGGGTGGTGGTCACAGCTCAGAAC-3'
		RH2B_reverse	5'-AATTAACCCTCACTAAAGGGACCATGCCACCCATTCCAAT-3'

Table S2. Summary of the stereology parameters used for the ganglion cell and photoreceptor topography analyses. SL = standard length, ∅ = diameter, DC = double cone, SC = single cone, CE = Schaeffer coefficient of error.

Ganglion cells

Species	Indiv	SL (cm)	Lens ∅ (mm)	Counting frame (μm x μm)	Grid (μm x μm)	CE
M. berndti	A	15.3	8.5	200 x 200	1700 x 1700	0.034
	B	16.5	9.4	200 x 200	1800 x 1800	0.037
M. violacea	A	12	6.5	150 x 150	1300 x 1300	0.032
	B	9.3	5.3	150 x 150	1050 x 1050	0.027
M. murdjan	A	15.7	8.8	200 x 200	1700 x 1700	0.035
M. pralinia	A	12.2	8.3	200 x 200	1600 x 1600	0.029
N. sammara	A	11.9	5.5	130 x 130	1150 x 1150	0.053
	B	14.3	6.7	130 x 130	1300 x 1300	0.056
S. spiniferum	A	19.7	7.1	150 x 150	1350 x 1350	0.045
	B	21.8	7.2	150 x 150	1400 x 1400	0.043
S. diadema	A	11.9	5.1	120 x 120	940 x 940	0.044
	B	11.7	5.0	120 x 120	1000 x 1000	0.048
S. rubrum	A	15.7	8.0	150 x 150	1450 x 1450	0.045
S. violaceum	A	15.6	6.6	150 x 150	1250 x 1250	0.041

Photoreceptors

Species	Indiv	SL (cm)	Counting frame DC (μm x μm)	Counting frame SC (μm x μm)	Grid (μm x μm)	CE DC	CE SC
M. berndti	C	10.2	150 x 150	300 x 300	1160 x 1160	0.030	0.037
M. violacea	B	9.3	150 x 150	300 x 300	1350 x 1350	0.028	0.050
	C	10.7	150 x 150	300 x 300	1250 x 1250	0.033	0.050
M. murdjan	B	14.7	300 x 300	450 x 450	1400 x 1400	0.035	0.057
	C	18.1	300 x 300	300 x 300	1750 x 1750	0.030	0.053
M. pralinia	A	12.2	300 x 300	300 x 300	1500 x 1500	0.035	0.063
N. sammara	A	11.9	130 x 130	260 x 260	1050 x 1050	0.027	0.033
	C	10.4	130 x 130	260 x 260	1000 x 1000	0.030	0.033
S. spiniferum	A	19.7	150 x 150	300 x 300	1370 x 1370	0.028	0.034
S. diadema	C	10.8	150 x 150	300 x 300	1080 x 1080	0.027	0.042
	D	11.7	150 x 150	300 x 300	950 x 950	0.039	0.049
S. rubrum	B	14	150 x 150	300 x 300	1200 x 1200	0.030	0.039
S. violaceum	A	15.6	200 x 200	400 x 400	1260 x 1260	0.025	0.038

Table S3. Summary of holocentrid transcriptomes, opsin gene mapping, and proportional opsin gene expression. *RH1* = rod opsin, *SWS2* = short-wavelength sensitive, *RH2* = rhodopsin-like. * Transcriptomes from Musilova et al. 2019.

Holocentrinae	ID	SRA Acc. No.	# raw (filter) reads	Rod RH1	Single cones SWS2A	RH2A-1	-2	-3	R	C	SWS2A	RH2A-1	-2	-3
Sargocentron spiniferum Lizard Island	F2	SAMN16670685	19,118,407 (14,126,874)	2,260,377	1,748	14,764 (1,064)	17611 (1,712)	496 (32)	98.5	1.5	5.1	36	57.9	1.1
Sargocentron rubrum Cairns Marine	F37	SAMN16670686	34,314,266 (23,032,335)	2,179,239	4,996	41,880 (3,190)	46779 (4,742)	-	95.9	4.1	5.4	38.1	56.6	-
Sargoceontron diadema Cairns Marine	F30	SAMN16670687	18,791,321 (13,400,810)	1,073,406	4,354	41,214 (1,528)	5866 (1970)	-	91.7	8.3	4.5	41.2	54.3	-
*Neoniphon sammara** Lizard Island	F3	SRX5060694	6,838,760 (6,264,679)	324,704	834	8,250 (90)	7,434 (70)	-	95.2	4.8	5.1	53.4	41.5	-
	F6	SRX5060695	4,506,938 (4,001,509)	170,140	422	4,240 (40)	3,942 (52)	-	95.2	4.8	4.9	41.3	53.7	-
	F10	SRX5060692	4,084,557 (3,464,740)	176,987	760	6,825 (116)	5,866 (66)	-	93.0	7.0	5.7	60.1	34.2	-
							Mean s.e.m.		94.9	5.1 1.0	5.1 0.2	45.0 3.9	49.7 3.9	- -

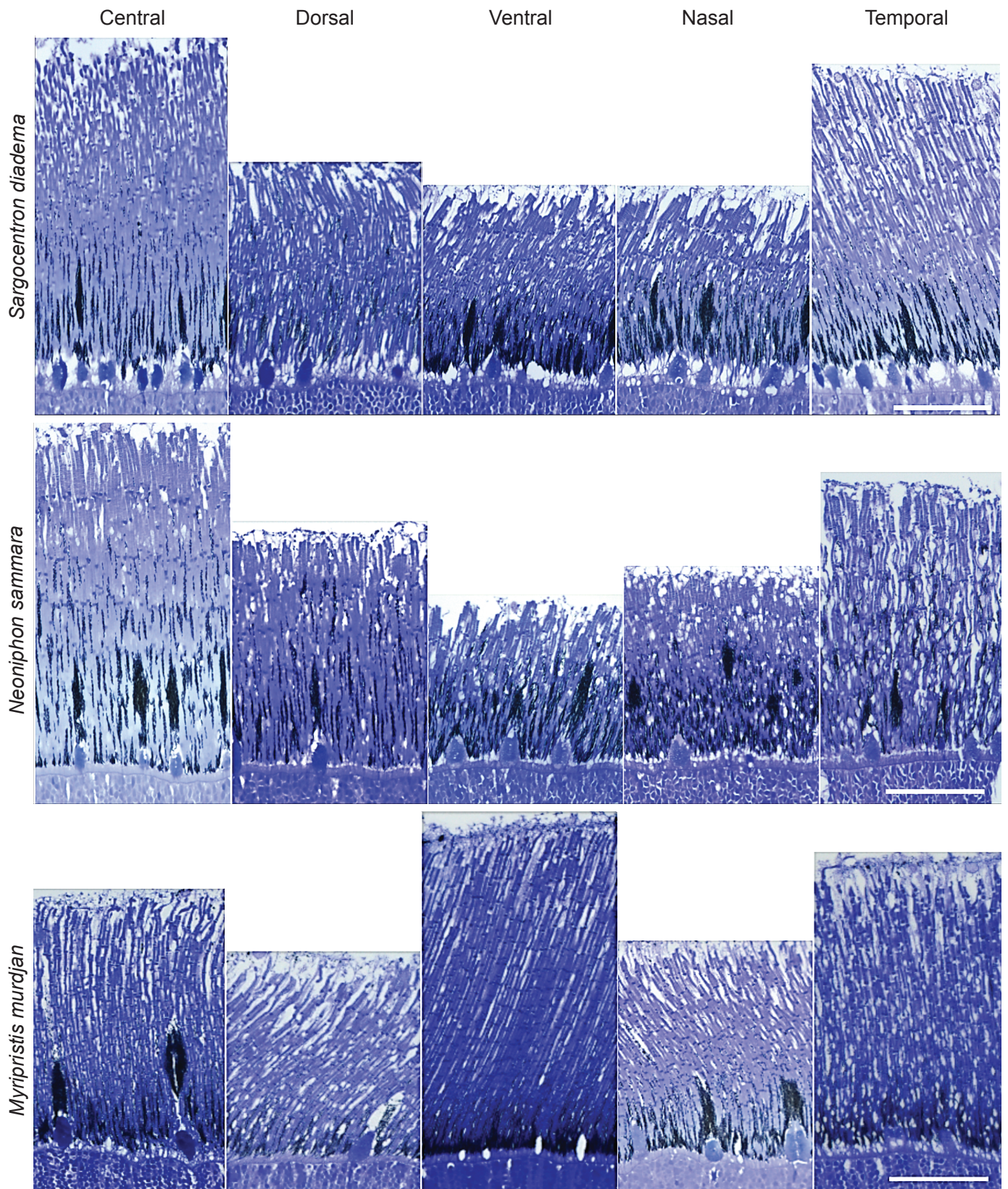| Myripristinae	ID	SRA Acc. No.	# raw (filter) reads	RH1	SWS2A	RH2B-1	R	C	SWS2A	RH2B-1
*Myripristis jacobus** Cape Verde	51	SRS4076665	17,244,006 (10,058,945)	912,891	386	7,363	99.1	0.9	4.9	95.1
	53	SRS4076643	32,578,163 (25,511,374)	1,926,830	732	4,910	99.7	0.3	12.8	87.2
*Myripristis berndti** Lizard Island	F7	SRS4076646	8,753,048 (6,071,712)	377,066	54	876	99.8	0.3	5.7	94.3
	F8	SRS4076637	6,812,942 (5,841,804)	541,184	98	1,682	99.7	0.3	5.4	94.6
	F11	SRS4076678	5,848,153 (5,271,695)	336,736	162	1,816	99.4	0.6	8.1	91.9
	F12	SRS4076668	4,810,822 (4,282,905)	362,974	126	1,972	99.4	0.6	5.9	94.1
Myripristis murdjan Lizard Island	F31	SAMN16670688	26042977 (19969212)	2,497,905	1,076	19,185	99.2	0.8	5.2	94.8
Myripristis violacea Lizard Island	F5	SAMN16670689	18679367 (13576628)	1,835,739	825	11,467	99.3	0.7	6.6	93.4
						Mean s.e.m.	99.4	0.6 0.1	6.8	93.2 0.9

Figure S1. Variation in the number of banks and thickness of the photoreceptor layer across the retina of three species of Holocentridae. Scale bar = 50 μm.
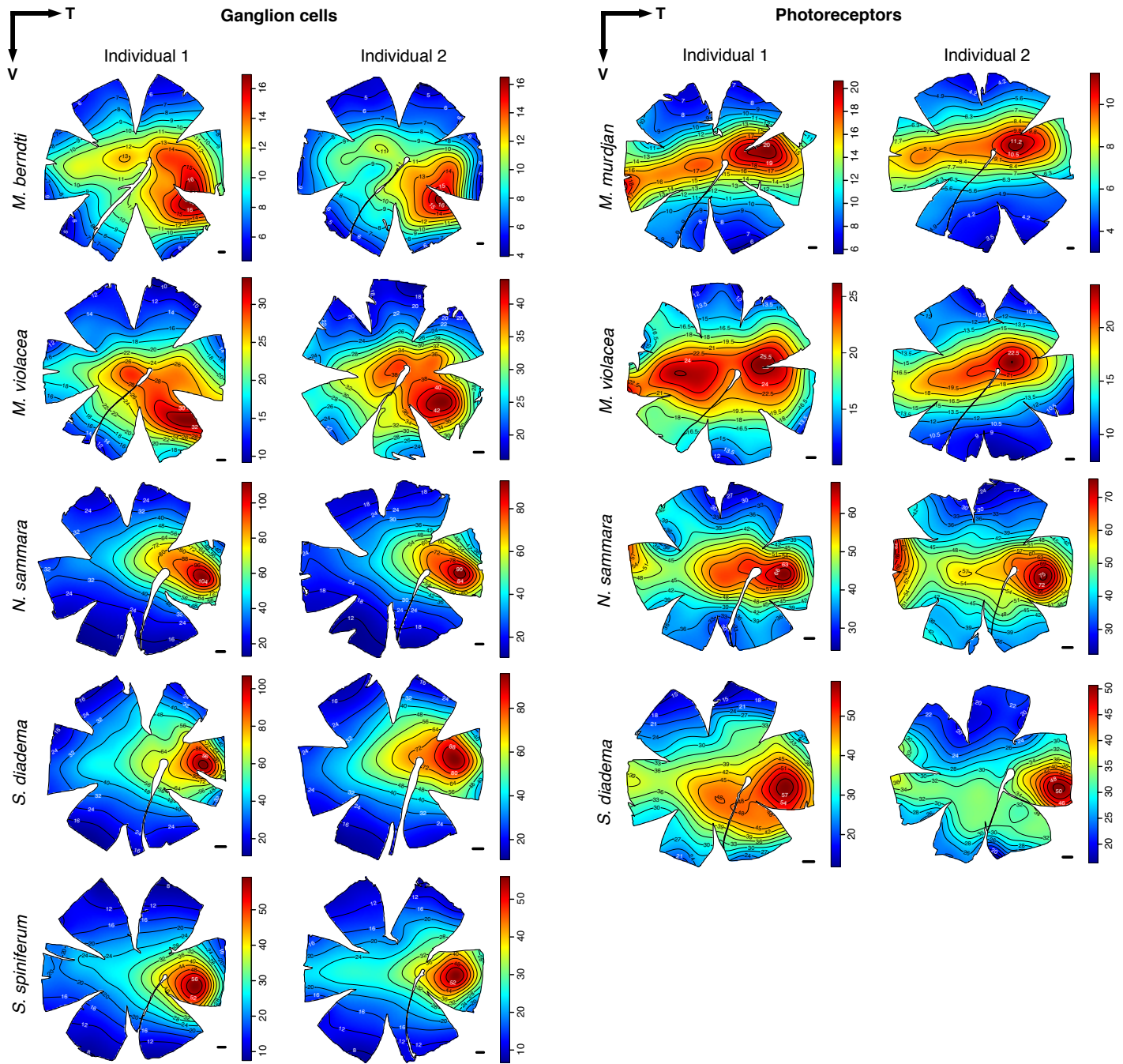
Figure S2. Intraspecific variability in the topographic distribution of ganglion cell and photoreceptor densities in the retinas of several species of Holocentridae. The black lines represent iso-density contours and values are expressed in densities x 10^2 cells/mm2. The black arrow indicates the orientation of the retinas. T = temporal, V = ventral. Scale bars = 1 mm.
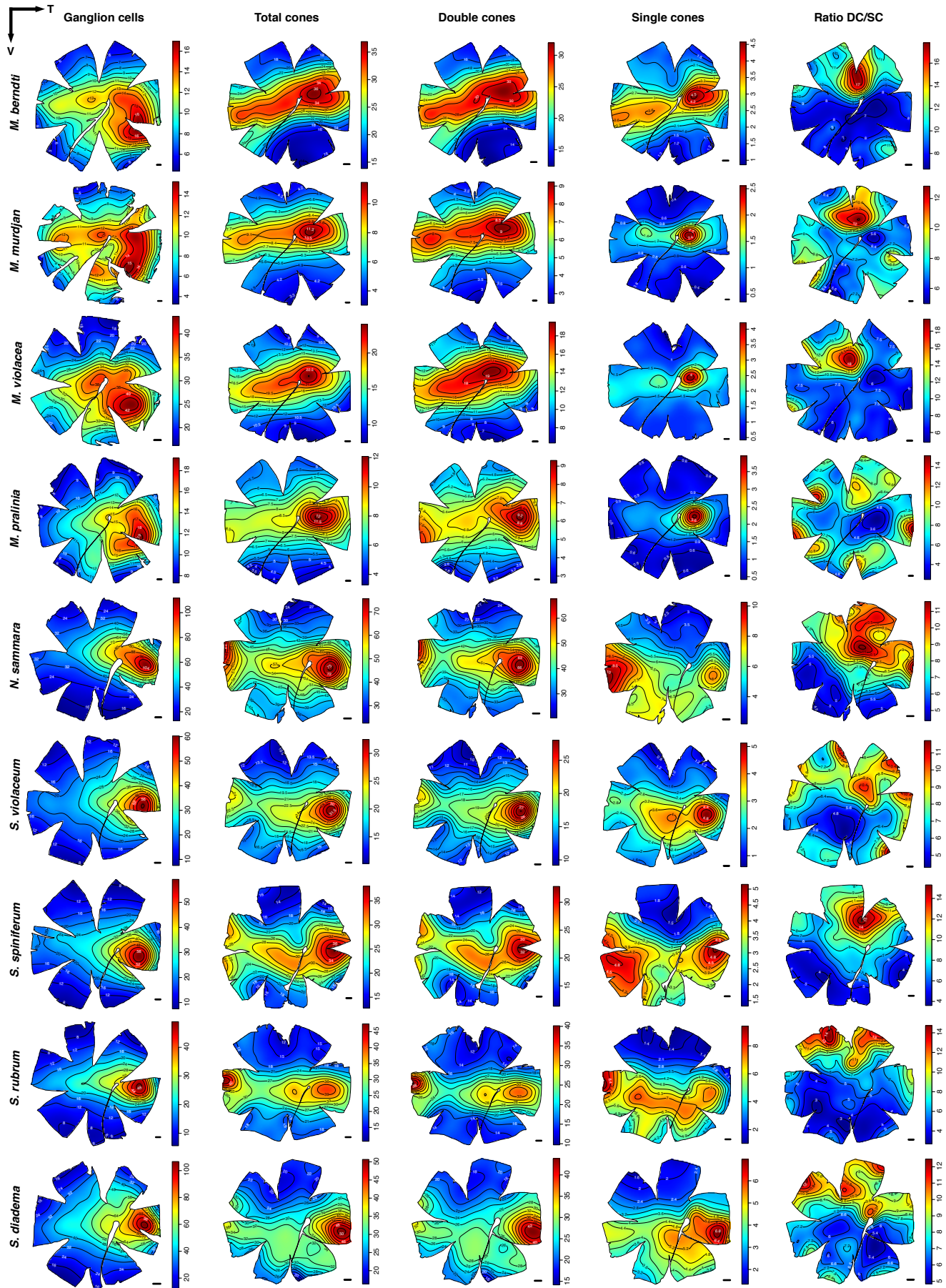
Figure S3. Topographic distribution of ganglion cell densities in the retinas of nine species of Holocentridae: The black lines represent iso-density contours and values are expressed in densities x 10^2 cells/mm^2, except for ratio DC/SC. The black arrow indicates the orientation of the retinas. T = temporal, V = ventral. Scale bars = 1 mm.