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SUPPLEMENTARY MATERIAL

corresponding to:

**Sexual dimorphism of AMH, DMRT1 and RSP01 localization
in the developing gonads of six anuran species**

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SUPPLEMENTARY TABLE S1

ANTIBODIES USED

Antibodies	Product number	Host species	Antigen	Concentration	Similarity between human and <i>X. laevis</i> protein
Anti-AMH	sc-20121 Santa Cruz Biotechnology	rabbit	N-terminus sequences between 1-115 aa of human AMH	1:50	53%
Anti-DMRT1	sc-98341 Santa Cruz Biotechnology	rabbit	C-terminus fragment 134-373 aa of human DMRT1	1:50	78%
Anti-RSPO1	ab73760 Abcam	rabbit	C-terminus domain 200-260 aa of human RSPO1	1:50	63%

SUPPLEMENTARY TABLE S2

IMMUNOLOCALIZATION OF AMH

Species	Sexually Undiff. Gonad		Testes		Ovary	
	Somatic cells	Germ cells	Somatic cells	Germ cells	Somatic cells	Germ cells
<i>Xenopus laevis</i>	+	-	+++	+	-	-
<i>Bombina bombina</i>	+	-	+++	+	-	-
<i>Bufo viridis</i>	-	-	+++	+	-	+
<i>Hyla arborea</i>	-	-	+++	++	-	-
<i>Rana arvalis</i>	+	-	+++	++	-	+
<i>Rana temporaria</i>	+	-	++	+	+	+

SUPPLEMENTARY TABLE S5

SAMPLING POINTS AND NUMBER OF ANIMALS USED PER EXPERIMENT

Species	Stages	N	N _U	N _M	N _F	
<i>Xenopus laevis</i>	49	11	11	-	-	
	50	10	10	-	-	
	53	9	1	4	4	
	55	12	-	7	5	
	60	9	-	4	5	
	64	8	-	4	4	
	66	5	-	3	2	
	sum	64	22	22	20	
	<i>Bombina bombina</i>	26	7	7	-	-
		30	9	9	-	-
34		10	-	6	4	
37		9	-	4	5	
40		9	-	4	5	
44		6	-	3	3	
46		9	-	3	6	
sum		59	16	20	23	
<i>Hyla arborea</i>		26	9	9	-	-
		30	12	12	-	-
	34	11	-	6	5	
	37	6	-	3	3	
	40	13	-	7	6	
	44	17	-	8	9	
	46	15	-	7	8	
	sum	83	21	31	31	
	<i>Bufo viridis</i>	26	10	10	-	-
		30	10	10	-	-
34		9	9	-	-	
37		14	14	-	-	
40		12	12	-	-	
44		15	1	6	8	
46		8	-	4	4	
sum		78	-	-	-	
<i>Rana arvalis</i>	26	10	10	-	-	
	30	7	7	-	-	
	34	10	10	-	-	
	37	8	-	4	4	
	40	11	-	5	6	
	44	12	-	5	7	
	46	17	-	8	9	
sum	75	27	22	26		
<i>Rana temporaria</i>	26	15	15	-	-	
	30	7	7	-	-	
	34	13	-	-	13	
	37	9	-	-	9	
	40	15	-	6	9	
	44	19	-	9	10	
	46	15	-	7	8	
	sum	93	22	22	49	

N-sum of used animals. N_U-number of individuals with undifferentiated gonads, N_M-number of males, N_F-number of females.

SUPPLEMENTARY TABLE S3

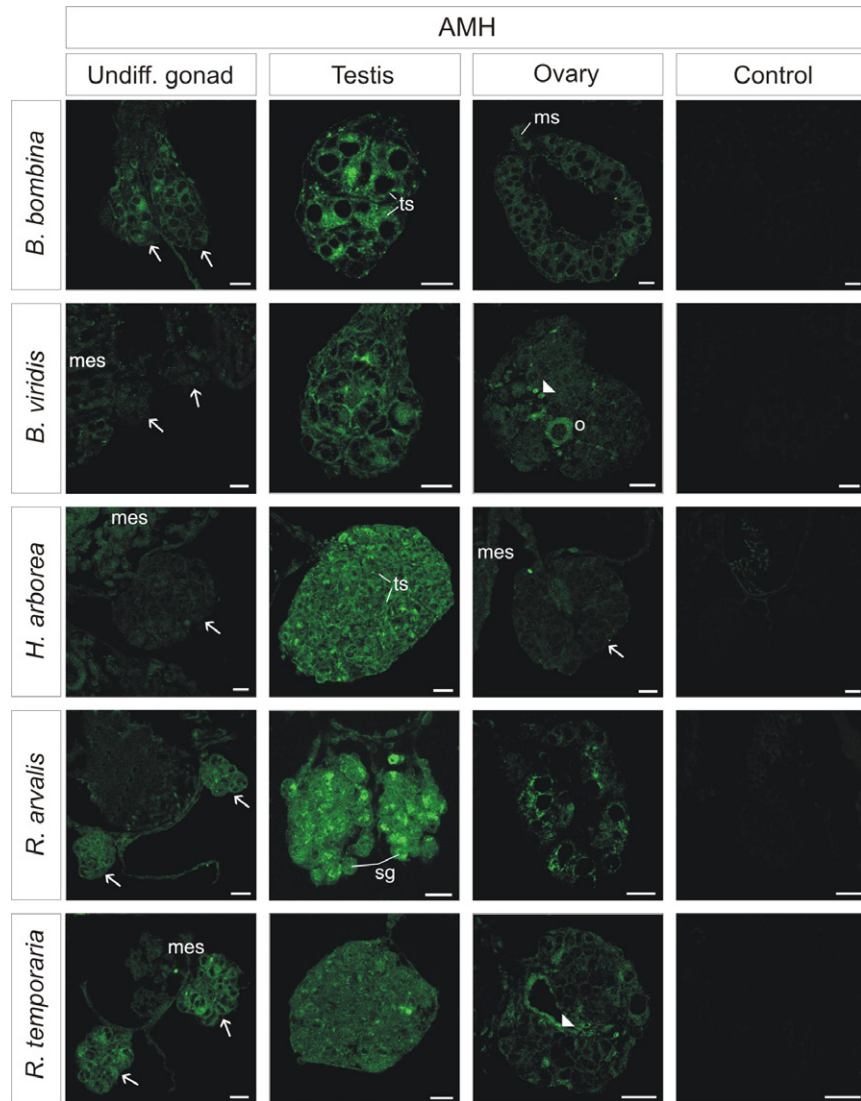
IMMUNOLOCALIZATION OF DMRT1

Species	Sexually Undiff. Gonad		Testes		Ovary	
	Somatic cells	Germ cells	Somatic cells	Germ cells	Somatic cells	Germ cells
<i>Xenopus laevis</i>	+	+	+++	+++	+	-
<i>Bombina bombina</i>	-	-	+++	+++	++	++
<i>Bufo viridis</i>	-	-	+++	+++	+	+
<i>Hyla arborea</i>	-	-	+++	+++	-	-
<i>Rana arvalis</i>	+	+	++	++	+	+
<i>Rana temporaria</i>	+	+	++	+	+	+

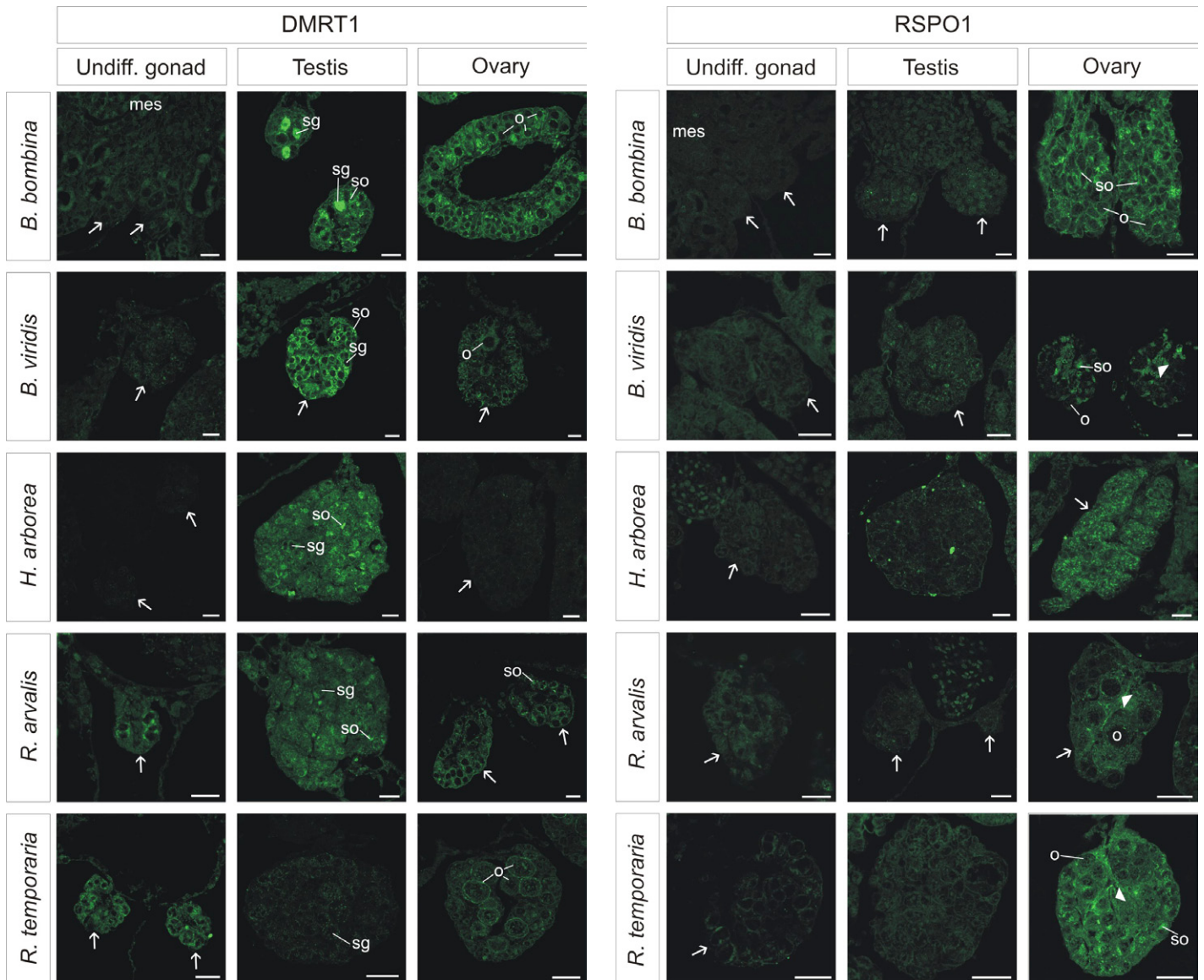
SUPPLEMENTARY TABLE S4

IMMUNOLOCALIZATION OF RSPO1

Species	Sexually Undiff. Gonad		Testes		Ovary	
	Somatic cells	Germ cells	Somatic cells	Germ cells	Somatic cells	Germ cells
<i>Xenopus laevis</i>	-	-	-	+	+++	+++
<i>Bombina bombina</i>	-	-	-	+	+++	+++
<i>Bufo viridis</i>	-	-	-	+	+++	+++
<i>Hyla arborea</i>	-	-	-	+	+++	+++
<i>Rana arvalis</i>	-	-	-	-	+++	+++
<i>Rana temporaria</i>	-	-	-	-	+++	+++



Supplementary Fig. S1. Immunolocalization of AMH in the undifferentiated gonads, testes and ovaries in anuran amphibians. Note the overall higher level of staining in testes vs. ovaries. Rightmost column - negative control incubated with rabbit serum. The gonads are pointed with arrows, *ms* - cells in the gonadal mesentery, *mes* - mesonephros, *o* - oogonial cells, *sg* - spermatogonial cells, *ts* - testis cords, arrow head - gonadal medulla. Scale bar, 40 μ m.



Supplementary Fig. S2 (Left). Immunolocalization of DMRT1 in the undifferentiated gonads, testes and ovaries in anuran amphibians. Note the clearly higher level of staining in testes vs. ovaries. The gonads are pointed with arrows, mes - mesonephros, o - oogonial cells, sg - spermatogonial cells, so - somatic cells. Scale bar, 40 μ m.

Supplementary Fig. S3 (Right). Immunolocalization of RSPO1 in the undifferentiated gonads, testes and ovaries in anuran amphibians. Note the overall higher level of staining in ovaries vs. testes. The gonads are pointed with arrows, mes - mesonephros, o - oogonial cells, so - somatic cells, arrow head - gonadal medulla. Scale bar, 40 μ m.