Supporting material for:
A molecular phylogeny of forktail damselflies (genus *Ischnura*) reveals a dynamic macroevolutionary history of female colour polymorphisms

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Table. S1. NCBI accession numbers of sequences used in this study to construct a time-calibrated phylogeny of *Ischnura* damselflies. Two mitochondrial (16S, COI) and three nuclear (D7, PMRT, H3) loci were used (See Methods). Missing sequences from specimens for which not all markers were sequenced are denoted with '--'. Samples sequenced for this study are marked in **bold**. Samples marked with * indicate representative sequences used for extemded phylogenetic analyses with PASTIS (see Supplementary Methods). Sequence data downloaded from NCBI GenBank come from published studies: 1 = Willink et al. (2019); 2 = Karube et al. (2012); 3 = Dijkstra et al. (2014), 4 = Bybee et al. (2008); 5 = Ferreira et al. (2014); 6 = Kim et al. (2014).

Taxon	Sample ID	16S	COI	D7	PMRT	H3
*Ischnura armstrongi**	BEA546[1]	MK874546	MK818631	MK874530	MK818716	MK818667
Ischnura armstrongi	**BEA575**	MT678014	MT680671	MT677959	MT665861	MT665812
Ischnura asiatica	**BEA276**	MT678017	MT680684	MT677961	MT665863	MT665815
*Ischnura asiatica**	BEA493[1]	MK874567	MK818665	MK874540	MK818725	MK818672
Ischnura asiatica	**BEA526**	MT678018	MT680685	MT677962	MT665864	MW556203
Ischnura asiatica	Kar1[2]	AB707553	AB708497	--	--	--
Ischnura aurora	**BEA528**	MT678020	MT680687	MT677963	--	--
*Ischnura aurora**	BEA548[1]	MK874550	MK818649	MK874533	MK818720	MK818673
Ischnura aurora	**BEA549**	MT678021	MT680690	MT677964	--	MT665817
Ischnura aurora	**BEA550**	MT678022	MT680689	MT677965	--	MT665818
Ischnura aurora	**BEA685**	MT678023	MT680688.2	MT677966	--	MT665819
Ischnura aurora	**BEA686**	MT678024	MT680691	MT677967	MT665865	MT665820
Ischnura aurora	Dij1[3]	KF369749	KF369414	KF370148	--	--
Ischnura aurora	**BEA123**	MT678019	MT680686	--	--	MT665816
Ischnura aurora	Kar1[2]	AB707554	AB708498	--	--	--
Ischnura aurora	Kar2[2]	AB707555	AB708499	--	--	--
Ischnura aurora	Kar3[2]	AB707556	AB708500	--	--	--
Ischnura aurora	Kar4[2]	AB707557	AB708501	--	--	--
Ischnura aurora	Kar5[2]	AB707558	AB708502	--	--	--
Ischnura aurora	Kar6[2]	AB707559	AB708503	--	--	--
*Ischnura barberi**	BEA104[1]	MK874570	MK818637	MK874521	MK818714	MK818674
Ischnura barberi	Byb1[4]	--	--	EU055231	--	EU055427
Ischnura capreolus	**BEA010**	MT678025	MT680676	MT677968	--	--
*Ischnura capreolus**	BEA411[1]	MK874555	MK818636	MK874536	MK818724	MK818675

Taxon	Sample ID	16S	COI	D7	PMRT	H3
Ischnura capreolus	**BEA641**	MT678026	MT680678	MT677969	MT665866	MT665821
Ischnura capreolus	**BEA727**	MT678027	MT680677	MT677970	--	MT665822
Ischnura capreolus	**BEA742**	MT678028	MT680679	MT677971	MT665867	MT665823
*Ischnura cardinalis**	BEA675[1]	MK874552	MK818633	MK874534	MK818722	MK818676
Ischnura cardinalis	**BEA688**	MT678029	MT680674	MT677972	--	MT665824
*Ischnura cervula**	BEA449[1]	MK874556	MK818653	MK874512	MK818728	MK818677
Ischnura cervula	**BEA450**	MT678030	MT680699	MT677973	MT665868	MT665825
Ischnura cervula	Fer1[5]	--	--	--	KM276622	--
*Ischnura cruzi**	BEA078[1]	MK874582	MK818661	MK874523	MK818734	MK818669
*Ischnura damula**	BEA087[1]	MK874560	MK818655	MK874509	MK818732	MK818678
*Ischnura demorsa**	BEA075[1]	MK874561	MK818656	MK874541	MK818729	MK818679
*Ischnura denticollis**	BEA217[1]	--	--	--	MK818738	MK818680
Ischnura denticollis	Fer1[5]	--	--	--	KM276603	--
*Ischnura elegans**	BEA494[1]	MK874571	MK818645	MK874517	MK818711	MK818681
Ischnura elegans	**BEA682**	MT678031	MT680720	MT677974	MT665869	MT665826
Ischnura elegans	**BEA689**	MT678032	MT680721	MT677975	MT665870	MT665827
Ischnura elegans	**BEA690**	MT678033	MT680722	MT677976	--	MT665828
Ischnura elegans	Dij1[3]	--	--	KF370149	--	--
Ischnura elegans	Kar1[2]	AB707560	AB708504	--	--	--
Ischnura elegans	Kar2[2]	AB707561	AB708505	--	--	--
Ischnura elegans	Kar3[2]	AB707562	AB708506	--	--	--
Ischnura elegans	Kim1[6]	KF256901	KF257118	--	--	--
*Ischnura erratica**	BEA326[1]	--	--	MK874545	--	MK818682
*Ischnura evansi**	BEA267[1]	MK874578	MK818644	MK874528	--	MK818683
*Ischnura ezoin**	BEA527[1]	MK874568	MK818666	MK874537	MW556205	MW556204
Ischnura ezoin	Kar1[2]	AB707523	AB708467	--	--	--
Ischnura ezoin	Kar2[2]	AB707524	AB708468	--	--	--
Ischnura ezoin	Kar3[2]	AB707525	AB708469	--	--	--
Ischnura ezoin	Kar4[2]	AB707526	AB708470	--	--	--

Taxon	Sample ID	16S	COI	D7	PMRT	H3
Ischnura ezoin	Kar5[2]	AB707527	AB708471	--	--	--
*Ischnura fluviatilis**	BEA043[1]	MK874579	MK818640	MK874519	MK818709	MK818684
Ischnura fluviatilis	**BEA056**	MT678034	MT680707	MT677977	MT665871	MT665829
Ischnura fluviatilis	**BEA643**	MT678036	MT680709	MT677978	MT665872	MT665831
Ischnura fluviatilis	**BEA738**	MT678037	MT680710	MT677979	MT665873	MT665832
Ischnura fluviatilis	**BEA642**	MT678035	MT680708	--	--	MT665830
*Ischnura fountaineae**	BEA479[1]	MK874572	MK818646	MK874516	MK818715	MK818685
*Ischnura foylei**	BEA673[1]	MK874575	MK818642	MK874532	MK818705	MK818686
*Ischnura gemina**	BEA344[1]	MK874559	MK818652	MK874542	MK818739	MK818687
Ischnura graellsii	**BEA425**	MT678038	MT680723	MT677980	MT665874	MT665833
*Ischnura graellsii**	BEA694[1]	MK874573	MK818647	MK874518	MK818713	MK818688
Ischnura graellsii	**BEA695**	MT678039	MT680724	MT677981	--	MT665834
Ischnura graellsii	**BEA696**	MT678040	MT680725	MT677982	--	MT665835
Ischnura hastata	**BEA051**	MT678041	MT680702	MT677983	MT665875	MT665836
*Ischnura hastata**	BEA705[1]	MK874584	MK818660	MK874526	MK818736	MK818689
Ischnura hastata	**BEA706**	MT678042	MT680703	MT677984	MT665876	MT665837
Ischnura hastata	Fer1[5]	--	--	--	KM276605	--
Ischnura hastata	Fer1[5]	--	--	--	KM276606	--
Ischnura hastata	Fer1[5]	--	--	--	KM276607	--
Ischnura hastata	Fer1[5]	--	--	--	KM276608	--
Ischnura hastata	Fer1[5]	--	--	--	KM276609	--
Ischnura hastata	Fer1[5]	--	--	--	KM276610	--
Ischnura hastata	Fer1[5]	--	--	--	KM276611	--
Ischnura hastata	Fer1[5]	--	--	--	KM276612	--
Ischnura hastata	Fer1[5]	--	--	--	KM276613	--
Ischnura hastata	Fer1[5]	--	--	--	KM276614	--
Ischnura hastata	Fer1[5]	--	--	--	KM276615	--
Ischnura hastata	Fer1[5]	--	--	--	KM276616	--
Ischnura hastata	Fer1[5]	--	--	--	KM276617	--

Taxon	Sample ID	16S	COI	D7	PMRT	H3
Ischnura hastata	Fer1[5]	--	--	--	KM276618	--
Ischnura hastata	Fer1[5]	--	--	--	KM276619	--
Ischnura hastata	Fer1[5]	--	--	--	KM276620	--
*Ischnura heterosticta**	BEA551[1]	MK874577	MK818641	MK874513	MK818707	MK818690
Ischnura heterosticta	**BEA552**	MT678044	MT680712	MT677985	MT665877	MT665838
Ischnura heterosticta	**BEA122**	MT678043	MT680711	--	--	--
Ischnura heterosticta	Kar1[2]	AB707563	AB708507	--	--	
*Ischnura intermedia**	BEA676[1]	MK874566	MK818663	MK874539	MK818727	MK818691
Ischnura intermedia	**BEA691**	MT678045	MT680680	MT677986	--	MT665839
*Ischnura kellicotti**	BEA105[1]	MK874565	MK818651	MK874515	MK818740	MK818692
Ischnura kellicotti	Fer1[5]	--	--	--	KM276621	
*Ischnura nursei**	Dij1[3]	KF369893	KF369538	KF370292	--	--
*Ischnura pamelae**	BEA451[1]	MK874551	MK818650	MK874535	MK818721	MK818693
*Ischnura perparva**	BEA084[1]	MK874557	MK818654	MK874508	MK818730	MK818671
*Ischnura posita**	**BEA083[1]**	MK874564	MT680692	MK874510	MK818731	MK818670
Ischnura posita	**BEA708**	MT678046	MT680697	MT677987	MT665878	MT665840
Ischnura posita	**BEA709**	MT678047	MT680701	MT677988	--	MT665841
Ischnura posita	**BEA710**	MT678048	MT680700	MT677989	--	MT665842
Ischnura posita	**BEA711[1]**	MT678049	MK818657	MT677990	MT665879	MT665843
Ischnura posita	**BEA712**	--	--	MT677991	MT665880	MT665844
Ischnura posita	Fer1[5]	--	--	--	KM276604	--
*Ischnura prognata**	BEA239[1]	MK874583	MK818659	MK874511	MK818737	--
*Ischnura pruinescens**	BEA353[1]	MK874554	MK818635	--	--	MK818694
Ischnura pumilio	**BEA375**	MT678050	MT680683	MT677992	MT665881	MT665845
Ischnura pumilio	**BEA525**	MT678051	MT680681	MT677993	MT665882	MT665846
*Ischnura pumilio**	**BEA718[1]**	MK874569	MK818664	MK874527	MT665883	MK818695
Ischnura pumilio	**BEA719[1]**	MT678052	MT680682	MT677994	MK818726	MT665847
Ischnura ramburii	**BEA155**	--	--	MT677995	MT665884	MT665848
*Ischnura ramburii**	BEA412[1]	MK874581	MK818638	MK874529	MK818708	MK818696

Taxon	Sample ID	16S	COI	D7	PMRT	H3
Ischnura ramburii	**BEA426**	MT678053	MT680705	MT677996	MT665885	MT665849
Ischnura rufostigma	**BEA132**	MT678054	MT680706	MT677997	--	--
*Ischnura rufostigma**	BEA465[1]	MK874580	MK818639	MK874538	MK818710	MK818697
Ischnura rufostigma	Kar1[2]	AB707564	AB708508	--	--	--
*Ischnura saharensis**	BEA481[1]	MK874574	MK818648	MK874525	MK818712	MK818698
Ischnura saharensis	**BEA430**	MT678055	MT680726	--	MT665886	MT665850
Ischnura senegalensis	**BEA474**	MT678056	MT680714	MT677998	MT665887	--
Ischnura senegalensis	**BEA477**	MT678057	MT680715	MT677999	MT665888	--
Ischnura senegalensis	**BEA478**	MT678058	MT680716	MT678000	MT665889	--
Ischnura senegalensis	**BEA488**	MT678059	MT680717	MT678001	MT665890	--
Ischnura senegalensis	**BEA490**	MT678060	MT680718	MT678002	MT665891	MT665851
Ischnura senegalensis	**BEA491**	MT678061	MT680719	MT678003	MT665892	--
Ischnura senegalensis	**BEA495**	MT678062	MT680713	MT678004	MT665893	MT665852
*Ischnura senegalensis**	BEA511[1]	MK874576	MK818644	MK874524	MK818706	MK818699
Ischnura senegalensis	Dij1[3]	--	--	KF370150	--	--
Ischnura senegalensis	Kar1[2]	AB707565	AB708509	--	--	--
Ischnura senegalensis	Kar2[2]	AB707566	AB708510	--	--	--
Ischnura senegalensis	Kar3[2]	AB707567	AB708511	--	--	--
Ischnura senegalensis	Kim1[6]	KF256888	KF257106	--	--	--
Ischnura sp.	**BEA545[1]**	MT678015	MK818632	MT677960	--	MT665813
Ischnura sp.*	**BEA670[1]**	MK874547	MT680672	MK874520	MK818717	MK818668
Ischnura sp.	**BEA671**	MT678016	MT680673	--	MT665862	MT665814
*Ischnura taitensis**	BEA674[1]	MK874553	MK818634	MK874531	MK818723	MK818700
Ischnura taitensis	**BEA687**	MT678063	MT680675	MT678005	MT665894	MT665853
*Ischnura ultima**	BEA554[1]	MK874585	MK818662	MK874522	MK818735	MK818701
Ischnura ultima	**BEA553**	MT678064	MT680704	MT678006	--	MT665854
*Ischnura verticalis**	**BEA111[1]**	MK874563	MK818658	MT678007	MK818733	MK818702
Ischnura verticalis	**BEA713**	MT678065	MT680698	MT678008	MT665895	MT665855
Ischnura verticalis	**BEA714**	MT678066	MT680695	MT678009	MT665896	MT665856

Taxon	**Sample ID**	**16S**	**COI**	**D7**	**PMRT**	**H3**
Ischnura verticalis	**BEA715**	MT678067	MT680696	MT678010	MT665897	MT665857
Ischnura verticalis	**BEA716**	MT678068	MT680693	MT678011	MT665898	MT665858
Ischnura verticalis	**BEA717**[1]	MT678069	MT680694	MK874514	MT665899	--
Pacificagrion lachrymosa	**BEA560**	MT678070	MT680670	MT678012	--	MT665859
*Pacificagrion lachrymosa**	BEA574[1]	MK874548	MK818629	MK874543	MK818718	MK818703
Pacificagrion sp.	**BEA547**	MT678071	MT680669	MT678013	--	MT665860
Pacificagrion sp.*	BEA576[1]	MK874549	MK818630	MK874544	MK818719	MK818704

Table S2. Character states and geographic range areas for 41 species of *Ischnura* damselflies included in this study. The variable 'State' indicates whether female-limited polymorphism (FP) occurs within a species, and if so, whether two (FP(D)) or three (FP(T)) female morphs are present, and in the case that a single morph exists, whether females are visually distinct from males in their colour pattern (heterochrome; FM(H)) or largely similar to males (androchrome; FM(A)). The variable 'StateBin' indicates if females are monomorphic (M), regardless of their colour pattern (heterochrome or androchrome), or if females are polymorphic (P), regardless of whether two or three female morphs co-occur. This was the response variable used in the geographic range size analysis (see Methods and Fig. 5). For each species, we report all countries of known occurrence, with names abbreviated according to the International Organization for Standardization (ISO). Whenever available we include lower-level administrative divisions, such as provinces, states and territories. For each species we calculate the total area of the geographic range by adding the areas of all administrative divisions where the species has been recorded, according to literature, online resources and museum collections listed under 'Sources'.

Species	**State**	**StateBin**	**Countries**	**Other divisions**	**Range area (Km^2)**	**Sources**
Ischnura armstrongi	FM(H)	M	ASM, WSM		3074	Marinov et al. (2015), Marinov (2020)*
Ischnura asiatica	FM(H)	M	CHN, HKG, JPN, KOR, PRK, RUS, TWN	Amur, Anhui, Beijing, Buryat, Chukot, Guangdong, Guangxi, Guizhou, Hebei, Heilongjiang, Henan, Hubei, Jiangsu, Jiangxi, Jilin, Kamchatka, Khabarovsk,Liaoning, Maga Buryatdan, Primor'ye, Sakha, Sakhalin, Shaanxi, Shandong, Shanghai, Sichuan,Tianjin, Xizang, Yevrey, Zabaykal'ye, Zhejiang	12002217	Haritonov and Malikova (1998), Ozono et al. (2012), Yu and Chen (2015), Seehausen and Fiebig (2016), Zhang et al. (2018), GBIF.org (2020),
Ischnura aurora	FP(D)	P	AUS, BGD, BTN, CHN, FJI, FSM, GUM, IDN, IND, JPN, LAO, LKA, MMR, MNP, NCL, NPL, PAK, PHL, PYF, SLB, TON, TWN, VUT, WSM	Andhra Pradesh, Arunachal Pradesh, Assam, Bihar, Fujian, Guangdong, Hainan, Himachal Pradesh, Kerala, Madhya Pradesh, Maharashtra, Manipur, Meghalaya, Nagaland, New South Wales, Northern Territory, Odisha, Ogasawara islands, Punjab, Queensland, Rajasthan, Ryukyu Islands, Sikkim, Tamil Nadu, Tasmania, Uttar Pradesh, Victoria, West Bengal, Western Australia	13907704	Rowe (1987), Ozono et al. (2012), Dow et al. (2020)
Ischnura barberi	FP(D)	P	MEX, USA	Arizona, California, New Mexico, Oklahoma, Texas, Utah	4058740	Paulson (2009)
Ischnura capreolus	FP(D)	P	ARG, BLZ, BRA, COL, CRI, ECU, SLV, GTM, GUF, GUY, HND, MEX, NIC, PAN, PER, PRY, SUR, TTO, VEN	Bahia, Espírito Santo, Mato Grosso, Pará, Pernambuco, Rio de Janeiro, Rio Grande do Sul, São Paulo	13113468	Heckman (2008), Kompier (2015), Vilela et al. (2017)

Species	State	StateBin	Countries	Other divisions	Range area (Km^2)	Sources
Ischnura cardinalis	FM(H)	M	PYF		3680	Marinov et al. (2019)
Ischnura cervula	FP(D)	P	CAN, MEX, USA	Alberta, Arizona, British Columbia, California, Colorado, Idaho, Montana, New Mexico, Nevada, Oregon, Saskatchewan, Utah, Washington, Wyoming	7288629	Paulson (2009)
Ischnura cruzi	FM(H)	M	COL		1136512	Heckman (2008), Realpe (2010)
Ischnura damula	FP(D)	P	CAN, USA	Alberta, British Columbia, Manitoba, Saskatchewan, Yukon, Arizona, Colorado, Nebraska, New Mexico, North Dakota, Texas, Utah, Wyoming	5820275	Paulson (2009)
Ischnura demorsa	FP(D)	P	MEX, USA	Arizona, Kansas, New Mexico, Oklahoma, Texas	3642413	Paulson (2009)
Ischnura denticollis	FP(D)	P	GTM, MEX, USA	Arizona, California, Idaho, Kansas, Nevada, New Mexico, Oklahoma, Oregon, Texas, Utah	5135142	Paulson (2009)
Ischnura elegans	FP(T)	P	ALB, AUT, BEL, BIH, BLR, BGR, CHE, CHN, CYP, CZE, DEU, DNK, ESP, EST, FIN, FRA, GBR, GRC, HRV, HUN, IDN, IND, IRL, IRN, ISR, ITA, JOR, JPN, KOR, LBN, LIE, LKA, LTU, LUX, LVA, MDA, MKD, MNE, MNG, MYS, NLD, NOR, NPL, PAK, PRK, POL, PSE, ROU, SRB, SVK, SVN, SWE, SYR, TUR, UKR	Himachal Pradesh, Jammu and Kashmir, Uttar Pradesh, West Bengal	23744568	Dijkstra and Lewington (2006), Dow (2010)
Ischnura erratica	FP(D)	P	CAN, USA	British Columbia, California, Oregon, Washington	1784487	Paulson (2009)
Ischnura evansi	FP(D)	P	AFG, ARE, BHR, EGY, IRN, IRQ, ISR, JOR, KAZ, KGZ, KWT, OMN, PSE, QAT, SAU, SDN, SYR, TJK, TKM, YEM		12210380	Marzoq (2005), Clausnitzer et al. (2013)*
Ischnura ezoin	FM(H)	M	JPN	Ogasawara islands	258	Ozono et al. (2012), Karube et al. (2020)
Ischnura fluviatilis	FP(D)	P	ARG, BOL, BRA, CHL, ECU, GUF, GUY, PRY, URY, VEN	Mato Grosso, Pará, Rio de Janeiro, Rio Grande do Sul, São Paulo	9375458	Heckman (2008), von Ellenrieder

Species	State	StateBin	Countries	Other divisions	Range area (Km^2)	Sources
						(2009), Kompier (2015)
Ischnura fountaineae	FP(D)	P	ARE, AZE, DZA, EGY, GEO, IRN, IRQ, ISR, JOR, KAZ, KGZ, LBR, LBY, MAR, OMN, PSE, QAT, RUS, SAU, SYR, TKM, TUN, TUR	Dagestan	14656465	Boudot et al. (2013), Schröter (2010), Seehausen et al. (2016)
Ischnura foylei	FP(D)	P	IDN	Jambi	49095	Kosterin (2015)
Ischnura gemina	FP(D)	P	USA	California	409615	Paulson (2009)
Ischnura graellsii	FP(T)	P	DZA, ESP, FRA, MAR, PRT, TUN		4023699	Dijkstra and Lewington (2006), Clausnitzer (2009)
Ischnura hastata	FM(H)	M	BLZ, COL, CRI, CUB, DOM, SLV, GTM, GUY, HND, MEX, NIC, PAN, TTO, USA, VEN	Alabama, Arizona, Arkansas, Connecticut, Florida, Georgia, Illinois, Indiana, Kansas, Kentucky, Louisiana, Massachusetts, Michigan, Mississippi, Missouri, Nebraska, New Jersey, New Mexico, New York, North Carolina, Ohio, Oklahoma, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Virginia, West Virginia	9301499	Esquivel (2006), Heckman (2008), Paulson (2012)
Ischnura heterosticta	FP(D)	P	AUS, FJI, IDN, NCL, PLW, PNG, SLB, TON, VUT	New South Wales, Northern Territory, Queensland, South Australia, Tasmania, Victoria, Western Australia	10113924	Huang and Reinhard (2012), Dow (2017a)
Ischnura intermedia	FM(H)	M	CYP, IRN, SYR, TKM, TUR	Adana, Adiyaman, Antalya, Batman, Burdur, Diyarbakir, Gaziantep, Hatay, Isparta, K. Maras, Karaman, Kilis, Mardin, Mersin, Mugla, Osmaniye, Sanliurfa, Siirt, Sirnak	2495078	Knifj et al. (2016)*
Ischnura kellicotti	FM(A)	M	USA	Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Illinois, Indiana, Kentucky, Louisiana, Maine, Massachusetts, Michigan, Mississippi, Missouri, New Hampshire, New Jersey, New York, North Carolina, Oklahoma, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Vermont, Virginia, West Virginia	3404330	Paulson (2012)

Species	State	StateBin	Countries	Other divisions	Range area (Km^2)	Sources
Ischnura nursei	FP(D)	P	BGD, IND, PAK	Chandigarh, Chhattisgarh, Madhya Pradesh, Maharashtra, Odisha, Punjab, Rajasthan, Tamil Nadu, Uttar Pradesh, Uttarakhand, West Bengal	3028389	Subramanian (2010)*
Ischnura pamelae	FM(H)	M	NCL		18775	Vick and Davies (1988), Kalkman (2020)
Ischnura perparva	FP(D)	P		Alberta, British Columbia, California, Colorado, Idaho, Montana, Nebraska, Nevada, New Mexico, North Dakota, Oregon, Saskatchewan, South Dakota, Utah, Washington, Wyoming	5624521	Paulson (2009)
Ischnura posita	FM(A)	M	BLZ, CAN, GTM, MEX, USA	Alabama, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Maine, Manitoba, Massachusetts, Michigan, Minnesota, Mississippi, Missouri, Nebraska, New Hampshire, New Jersey, New York, Newfoundland and Labrador, North Carolina, Nova Scotia, Ohio, Oklahoma, Ontario, Québec, Pennsylvania, Rhode Island, South Carolina, Tennessee, Texas, Vermont, Virginia, Wisconsin, West Virginia	10273446	Paulson (2012)
Ischnura prognata	FM(H)	M	USA	Alabama, Florida, Georgia, Louisiana, Missouri, North Carolina, South Carolina, Tennessee, Texas, Virginia, West Virginia	1915019	Paulson (2012)
Ischnura pruinescens	FM(H)	M	AUS, IDN	Northern Territory, Papua, Queensland	3392025	Theischinger and Hawking (2006), Dow (2017b)
Ischnura pumilio	FP(D)	P	AFG, ALB, AND, ARM, AUT, AZE, BEL, BIH, BLR, BGR, CHE, CHN, CYP, CZE, DEU, DNK, ESP, EST, FIN, FRA, GBR, GRC, HRV, HUN, IRL, IRN, ISR, ITA, JOR, KAZ, KGZ, LBN, LIE, LTU, LUX, LVA, MAR, MDA, MKD, MNE, MNG, NLD, NOR,	Adygey, Arkhangel'sk, Astrakhan', Bashkortostan, Belgorod, Bryansk, Chechnya, Chelyabinsk, Chuvash, City of St. Petersburg, Dagestan, Ingush, Ivanovo, Kabardin-Balkar, Kaliningrad, Kalmyk, Kaluga, Karachay-Cherkess, Karelia, Khanty-Mansiy, Kirov, Komi, Kostroma, Krasnodar, Kurgan, Kursk, Leningrad, Lipetsk, Mariy-El, Mordovia, Moscow City, Moskva, Murmansk, Nei Mongol, Nenets, Nizhegorod,	22384600	Dijkstra and Lewington (2006), Boudot (2014)

Species	State	StateBin	Countries	Other divisions	Range area (Km^2)	Sources
			POL, PRT, ROU, RUS, SRB, SVK, SVN, SWE, SYR, TJK, TKM, TUN, TUR, UKR, UZB	North Ossetia, Novgorod, Orel, Orenburg, Penza, Perm', Pskov, Rostov, Ryazan', Samara, Saratov, Shanxi, Smolensk, Stavropol', Sverdlovsk, Tambov, Tatarstan, Tula, Tver', Tyumen', Udmurt, Ul'yanovsk, Vladimir, Volgograd, Vologda, Voronezh, Yamal-Nenets, Yaroslavl'		
Ischnura ramburii	FP(D)	P	BHS, BLZ, BRA, CHL, COL, CRI, CUB, CYM, DOM, ECU, SLV, GLP, GTM, GUF, HND, HTI, JAM, MEX, MTQ, NIC, PAN, PER, PRI, PRY, SUR, TTO, VEN	Alabama, Arizona, Arkansas, Connecticut, Delaware, District of Columbia, Florida, Georgia, Illinois, Kentucky, Louisiana, Maine, Massachusetts, Mississippi, Missouri, New Jersey, New York, North Carolina, Oklahoma, Pennsylvania, Rhode Island, South Carolina, Texas, Virginia	19316199	Paulson (2017)
Ischnura rufostigma	FP(D)	P	BGD, HKG, LAO, MMR, NPL, THA, VNM	Assam, Bihar, Guangdong, Guizhou, Himachal Pradesh, Hunan, Madhya Pradesh, Manipur, Meghalaya, Nagaland, Sichuan, Uttarakhand, West Bengal, Yunnan	4199316	Mitra (2010), Sanmartin-Villlar et al. (2016)
Ischnura saharensis	FP(D)	P	DZA, LBY, MAR, MRT, NER, TUN		6717192	Samraoui and Dijkstra (2010)
Ischnura senegalensis	FP(D)	P	AFG, AGO, ARE, BEN, BFA, BGD, BRN, BWA, CMR, COD, CPV, DJI, EGY, ESH, GAB, GHA, GMB, GNB, GNQ, HKG, IDN, IND, IRN, IRQ, ISR, JOR, JPN, KEN, KHM, LAO, LBR, LBY, LKA, LSO, MAC, MDG, MLI, MMR, MOZ, MRT, MUS, MWI, MYS, NAM, NER, NGA, OMN, PAK, PHL, PLW, PNG, PSE, REU, RWA, SAU, SDN, SEN, SGP, SLE, SOM, SSD, SWZ, SYC, TCD, TGO, THA, TKM, TLS, TWN, TZA, UGA, UZB, VNM, YEM, ZAF, ZMB, ZWE	Nei Mongol	41440050	Ozono et al. (2012), Sharma and Clausnitzer (2016)
Ischnura sp.	FM(H)	M	ASM		222	Marinov et al. (2015)

Species	State	StateBin	Countries	Other divisions	Range area (Km^2)	Sources
Ischnura taitensis	FM(A)	M	PYF		3680	Marinov et al. (2019)
Ischnura ultima	FP(D)	P	ARG, CHL		3537305	von Ellenrieder and Garrison (2007), Heckman (2008)
Ischnura verticalis	FP(D)	P	CAN, USA	Arkansas, Colorado, Connecticut, Delaware, District of Columbia, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Maine, Massachusetts, Michigan, Minnesota, Missouri, Montana, Nebraska, New Brunswick, New Hampshire, New Jersey, New Mexico, New York, Newfoundland and Labrador, North Carolina, North Dakota, Nova Scotia, Ohio, Oklahoma, Ontario, Pennsylvania, Québec, Rhode Island, South Carolina, South Dakota, Tennessee, Texas, Vermont, Virginia, West Virginia, Wisconsin, Wyoming	8679697	Paulson (2009), Paulson (2012)
Pacificagrion lachrymosa	FM(H)	M	ASM		222	Marinov et al. (2015)
Pacificagrion sp.	FM(H)	M	ASM		222	Marinov et al. (2015)

*Classification of female-colour states complemented by observation of field or museum specimens, as described in Willink et al. (2019)

Table S3. Geographic range areas for 23 species of *Ischnura* damselflies not included in this or previous studies (Robinson and Allgeyer, 1996; Mattern and van Gossum, 2008; Sánchez-Guillén et al., 2020). For each species, we report countries of known occurrence, with names abbreviated according to the International Organization for Standardization (ISO). We include additional information on geographic range such as names of islands, provinces or altitudinal range, where available. Geographic range information comes from literature listed under 'Sources'. Two species, *I. ordosi* and *I. rubella*, marked as "doubtful" in the World Odonata List (Schorr and Paulson, 2020) were excluded.

Species	**Countries**	**Other range information**	**Sources**
Ischnura acuticauda	PNG	From middle to high elevations (1800-2900 m.a.s.l.)	Kalkman and Orr (2013)
Ischnura albistigma	WSM	Upolo Island and Tutuila Island	Fraser (1953)
Ischnura ariel	IDN	Papua, province, known from a few middle elevation sites (1740-1800 m.a.s.l.)	Kalkman and Orr (2013)
Ischnura armeniaca	IDN	Papua province, endemic to highlands (3200-3900 m.a.s.l.)	Kalkman and Orr (2013)
Ischnura auricolor	WSM	Upolo Island	Fraser (1927)
Ischnura buxtoni	WSM	Upolo Island	Fraser (1953)
Ischnura chromostigma	WSM	Tutuila Island	Fraser (1927)
Ischnura filosa	MDG	Madagascar Island	Fraser (1949a)
Ischnura haemastigma	WSM	Upolo Island	Fraser (1953)
Ischnura inarmata	IND	Kashmir	Calvert (1898)
Ischnura isoetes	IDN	Papua province, from high elevations (2500-3450 m.a.s.l.)	Kalkman and Orr (2013)
Ischnura jeanyvesmeyeri	PYF	Endemic to the island of Raivavae, Austral Islands	Englund and Polhemus (2010)
Ischnura lorentzi	IDN	Papua province, known from a single female	Kalkman and Orr (2013)
Ischnura luta	MNP	Endemic to island of Rota	Polhemus et al. (2000)
Ischnura mahechai	COL	Known from a highland lake (3600 m.a.s.l.)	Machado (2012)
Ischnura oreada	IDN	Papua province, endemic to highlands (3200-3700 m.a.s.l.)	Kalkman and Orr (2013)
Ischnura rufovittata	BOL		Heckman (2008)
Ischnura rurutana	PYF	Endemic to the island of Rurutu, Austral Islands	Englund and Polhemus (2010)
Ischnura sanguinostigma	WSM	Upolo Island	Fraser (1953)

Species	Countries	Other range information	Sources
Ischnura stueberi	IDN	Papua province, known from several lowland localities	Kalkman and Orr (2013)
Ischnura thelmae	PYF	Endemic to the island of Rapa, Austral Islands	Englund and Polhemus (2010)
Ischnura vinsoni	MUS	Mauritius Island	Fraser (1949b)
Ischnura xanthocyane	IDN	Papua province, endemic to highlands (3000-3500 m.a.s.l.)	Kalkman and Orr (2013)

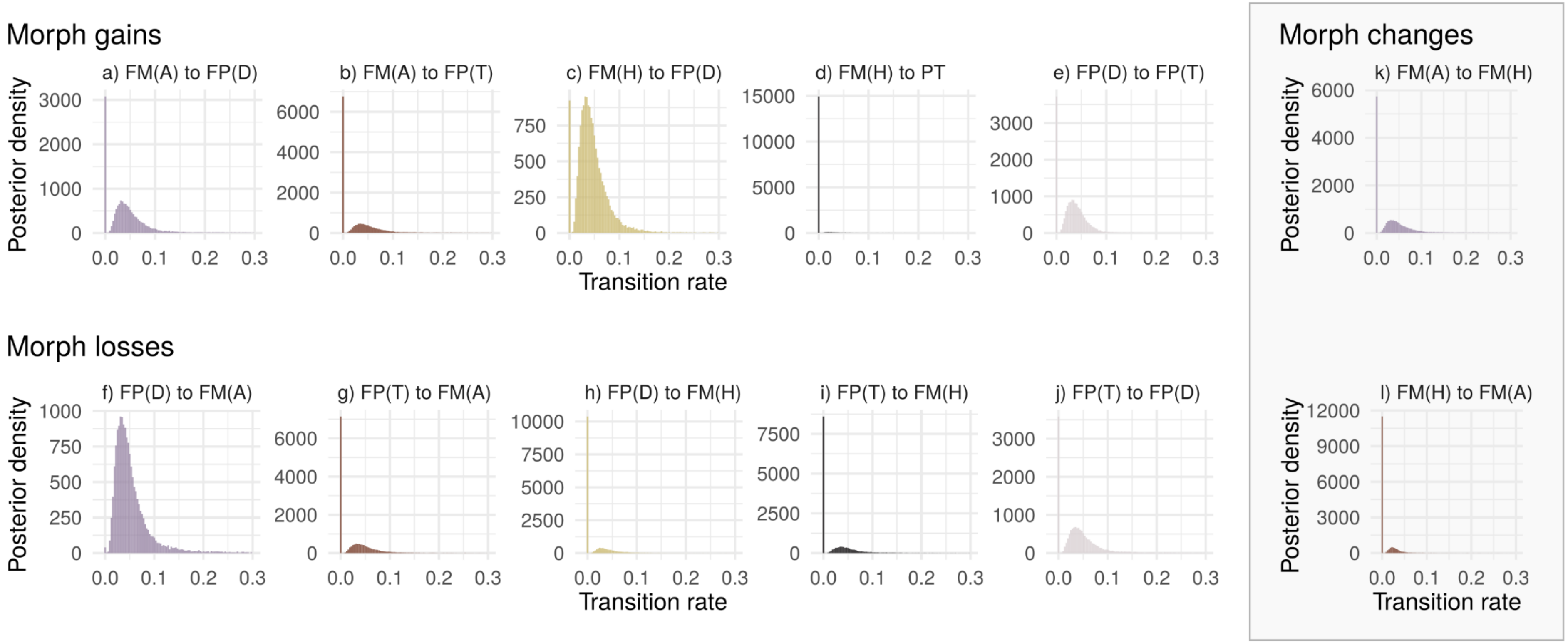

Figure S1. Posterior distribution of transition rate parameters in a multistate, reversible-jump (RJ) MCMC model of the evolution of female colour and female colour polymorphisms in *Ischnura* damselflies. The RJ model samples the parameter number and their values proportionally to their posterior probability. As a result, in each posterior sample a transition rate parameter may be equal to zero, or it may be included in a rate category with an estimated rate. Transition rate parameters may therefore have a bimodal posterior distribution, with a peak at zero and a peak at the mode of the posterior samples in which the estimated parameter is greater than zero. Transitions can occur between four character states: FM(A) = Monomorphic females with colour pattern similar to males (androchrome females), FM(H) = Monomorphic females with colour pattern markedly different from males (heterochrome females), FP(D) = Polymorphic females with two female morphs (one androchrome, one heterochrome), FP(T) = Polymorphic females with three female morphs (one androchrome, two heterochrome). **(a-e)** Transitions from FM(A) and FM(H) to either FP(D) or FP(T), and from FP(D) to FP(T) involve an evolutionary gain of novel female morphs. **(f-j)** Transitions away from FP(D) or FP(T) to FM(A) and FM(H), and from FP(T) to FP(D) occur if female morphs are lost. **(k-l)** Transitions in the grey box represent evolution of female or male colouration without the evolution of female polymorphisms.

Supporting Materials and Methods

Expanded phylogeny reconstruction

A recent study explored the origin and evolutionary dynamics of female colour polymorphism in *Ischnura* using a single MCC tree and stochastic character mapping (Sánchez-Guillén et al., 2020). The MCC tree incorporated nine species (*I. abyssinica, Ischnura sp. "a", I. aralensis, I. chingaza, I. cyane, I. forcipata, I. genei, I. indivisa, I. rubilio*) and two subspecies (*I. elegans ebneri* and *I. posita atezca*) that were additional to the subset used in this study. Sánchez-Guillén et al. (2020) inferred the most recent common ancestor (MRCA) of *Ischnura* as female-polymorphic (FP).

Sánchez-Guillén et al. (2020) conducted phylogenetic inference based on two mitochondrial and one nuclear marker, none of which were used in the present study due to poor taxonomic coverage. In order to investigate if our conflicting conclusions could be explained by the taxa missing in the present study, we used a Phylogenetic Assembly with Soft Taxonomic Inferences (PASTIS) approach (Thomas et al., 2013) in R v.3.6.1 (R Core Team 2020) to incorporate species lacking genetic data at the tree inference stage. As our MCC tree was inferred under a birth-death model, assuming each lineage is a species, we did not include the two subspecies sampled by Sánchez-Guillén et al. (2020) in these analyses. We used the PASTIS method to combine our MCC tree, inferred using StarBEAST2, sequence data (limited to one representative individual per species) and a set of taxonomic statements to guide the placement of the taxa lacking sequence data.

Each species, excluding *I. abyssinica* and *I. aralensis*, was treated as a separate clade, and missing taxa were assigned a number of sister species among which placement was random. We drew sister taxa assignments from the MCC tree in Sánchez-Guillén et al. (2020). In this guide tree, *I. abyssinica* and *I. aralensis* were inferred as sister species with 100% support. The phylogenetic placement of this two-species clade was uncertain in Sánchez-Guillén et al. (2020), so we restricted its placement in our extended tree to the least inclusive clade with support greater than 80%. Consequently, the sister taxa *I. abyssinica* and *I. aralensis* were randomly assigned within the clade in our MCC tree including *I. barberi, I. nursei, I. evansi, I. fluviatilis, I. fountaineae, I. saharensis, I. elegans, I. graellsii, I. rufostigma, I. ramburii, I. hetersticta, I. foylei* and *I. senegalensis* (see Fig. 2).

In the guide MCC tree (Sanchez-Guillén et al., 2020), three of the missing species (*I. cyane, I. indivisa* and *I. sp. "a"*) form a monophyletic clade with *I. caprelous,* and another species (*I. chingaza*) forms a monophyletic clade with *I. cruzi*. These clades are well supported at 100% support. Therefore, in our model we constrained the former three and latter one missing species to be sister taxa to *I. capreolus* and *I. cruzi* respectively. Equally, the missing species *I. rubilio* was inferred as sister to *I. aurora* at 100% in the guide MCC tree (Sanchez-Guillén et al., 2020). As Sánchez-Guillén et al. (2020) did not sample *I. pamelae* (inferred as sister to *I. aurora with* 100% support in the MCC tree from this study), *I. rubilio* was conservatively constrained to fall within the clade including *I. aurora* and *I. pamelae* in our PASTIS model. The placement of the missing species *I. forcipata* in a monophyletic clade with *I. asiatica, I. pumilio, I. ezoin* and *I. intermedia* was well-supported (99%) in the guide MCC tree, although the exact placement was uncertain (Sanchez-Guillén et al., 2020). Therefore, we placed this species randomly within this clade in our model.

Finally, we constrained the missing species *I. genei* to the clade in our MCC tree including *I. nursei, I. evansi, I. fluviatilis, I. fountaineae, I. saharensis, I. elegans, I. graellsii, I. rufostigma, I. ramburii, I. hetersticta, I. foylei* and *I. senegalensis.* In Sánchez-Guillén et al. (2020), *I. genei* falls within a less inclusive clade with relatively high support (82%). Yet, this less inclusive clade does not appear in the MCC tree from the present study. We thus assigned a random phylogenetic placement of *I. genei* to a more inclusive clade that is shared by Sánchez-Guillén et al. (2020) and the present study. However, this clade is not strongly supported (< 80%) in Sánchez-Guillén et al. (2020), and has moderate support in this study (79%).

Bayesian assessment of the placement of missing taxa was then conducted using MrBayes v.3.2.7 (Ronquist et al., 2012). Most of the default settings outlined in the PASTIS template were used in the phylogenetic model and MCMC algorithm implemented in MrBayes. We placed a birth-death prior on the species tree and fixed extinction rates to zero. We used an independent gamma rate (IGR) relaxed clock model and the expected variability in branch lengths was drawn from an exponential prior with a mean of 10. We also placed an exponential prior with a mean of 1 on the speciation rate. We specified a molecular evolution model with 5 partitions, one for each locus. Each partition had its own generalized time reversible (GTR) substitution model under default priors. Site-rate heterogeneity was modelled as gamma distributed with four rate categories for all loci, except for D7, in which the proportion of invariant sites was estimated. A unique rate multiplier was set for each partition, except for the two mitochondrial markers (16S and COI), which were linked. We ran a single Markov Chain Monte Carlo (MCMC) algorithm for 50 million iterations with a sampling frequency of 10000 and disposed of the first 10 million samples as burnin. Stationarity and convergence checks of MCMC runs were performed as per the main text (see Materials and Methods).

Out of the nine additional species included in this analysis, two (*I. rubilio* and *I. forcipata*) were classified as female-monomorphic with heterochrome females (FM(H)) according to Sánchez-Guillén et al. (2020). Six species (*I. aralensis*, *I. abyssinica*, *I. indivisa*, *I. sp. a*, *I. cyane* and *I. chingaza*) were classified as female-polymorphic with dimorphic females (FP(D)) (Sánchez-Guillén et al. 2020). However, we note that for *I. cyane* and *I. chingaza* this classification was based on unpublished observations, and a taxonomic study cited by Sánchez-Guillén et al. (2020) indicates that colour polymorphisms have not been found so far in these species (Realpe, 2010). This also is the case for *I. cruzi*, which we classified as FM(H), but was classified as FP(D) in Sánchez-Guillén et al. (2020), based on unpublished observations. For this analysis we assumed *I. cruzi* to be FP(D) as in Sánchez-Guillén et al. (2020). *I. genei,* the last missing taxon, was classified as female-polymorphic with trimorphic females (FP(T)) (Sánchez-Guillén et al., 2020).

There are four other conflicts in female-colour classification between the present study and Sánchez-Guillén et al. (2020). We classified *I. aurora* as FP(D), as androchrome females are described and photographed, albeit reportedly rare, in Ozono et al. (2012). In contrast, this species was classified as FM(H) in Sánchez-Guillén et al. (2020). *I. evansi* was classified as FP(T) by Sánchez-Guillén et al. (2020) and FP(D) in the present study, as we could not ascertain if the three phenotypic forms in Marzoq (2005) represented one androchrome and two heterochrome morphs or one androchrome and one heterochrome morph sampled in two developmental colour phases. Sánchez-Guillén et al. (2020) classified *I. posita* as FM(H). Here, we classified it as FM(A), according to Paulson (2012), who described females as similar in colour pattern as males, but with postocular spots and thorax stripes pale to blue rather than light green, and developing more pruinosity with age than males do. Finally, *I. heterosticta* was classified as FM(H) in Sánchez-Guillén et al. (2020), citing Huang et al. (2012). However, Huang et al. (2012) actually describe two distinct female morphs, an andro-gynochrome morph that starts out with a male-like colour pattern and changes to a green and then grey colour with age, and a gynochrome (same as heterochrome) morph that does not undergo this ontogenetic colour change.

Of these four conflicts, two (*I. aurora* and *I. heterosticta*) affect whether a species is classified as female polymorphic (FP) or female monomorphic (FM), whereas the other two only relate to the extent of sexual dimorphism in a FM taxon (*I. posita*), or the number of female morphs in a female-polymorphic species (*I. evansi*). As the analysis in Sánchez-Guillén et al. (2020) only considered whether females were monomorphic (FM) or polymorphic (FP), and we re-classified *I. cruzi* as FP(D), only 2 out of 50 species have a directly conflicting female-colour classification between this supplementary analysis and the one in Sánchez-Guillén et al. (2020).

We then used the Multistate method in BayesTraits v.3.0 (Pagel et al., 2004) to infer female-colour character states at ancestral nodes, given the extended *Ischnura* phylogeny and the female-colour character states of extant species. We ran the Multistate model using a sample of 1000 trees from one independent run of the phylogenetic assembly analyses and kept all phylogenetic model and MCMC algorithm parameters the same as per the analyses from the main text (see Materials and Methods). Ancestral character states were mapped onto the maximum clade credibility (MCC) tree, drawn from the MrBayes output.

Results

The results from this analysis were qualitatively similar to the main ancestral state reconstruction of this study, and the MRCA of *Ischnura* was again inferred as being FM(H). However, the female-colour state was inferred with a slightly lower mean posterior probability (PP) than in the main analyses of 66% (Fig. S2). Equally, the probability that the ancestor was FP(D) increased to 33%. We note that 7 out of the 9 previously missing taxa included in these analyses are either FP(D) or FP(T), likely driving the increase in probability of a FP ancestor in *Ischnura.* However, FM species may be just as numerous as FP species across the genus (Willink et al. 2019), and they remain under-sampled as they are more likely to occupy confined geographic ranges such as remote islands (see Discussion).

Figure S2. Ancestral state reconstruction plotted on the extended *Ischnura* MCC tree. Nine additional species (labelled with asterisks) were added using soft taxonomic inferences. Ancestral state reconstruction was conducted on a posterior sample of 1000 trees. For each tree, the positions of missing taxa were constrained to specific clades, based on previous phylogenetic inference, and randomized within those clades (see Supporting Methods). We plot these results on a single MCC tree for simplicity. Extant morph states are represented by circles at tips and reconstructed ancestral states are represented by piecharts at nodes (female-heterochromatic only, green; female-andromorphic only, blue; female-dimorphic, red; female-trimorphic, pink). Piecharts show the mean posterior probability for each morph state and are plotted on the consensus tree.

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