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TECHNICAL NOTE

Microsatellite markers for species of the genus *Dionda* (Cyprinidae) from the American southwest

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Abstract Thirty-eight microsatellite markers were developed from an enriched genomic DNA library of the cyprinid fish (minnow) *Dionda episcopa*. The microsatellites include 31 perfect-repeat motifs (29 dinucleotide, 1 trinucleotide, and 1 tetranucleotide) and 7 imperfect-repeat dinucleotide motifs. The microsatellite primers were used to amplify microsatellites from five related congeners: *D. argentosa*, *D. diaboli*, *D. episcopa*, *D. nigrotaeniata*, and *D. serena*. One species (*D. diaboli*) is listed as threatened and critically imperiled and two species (*D. argentosa* and *D. serena*) are listed as imperiled; the conservation status of *D. nigrotaeniata* is unknown. The number of experimentally tractable microsatellite markers varied from 28 for *D. diaboli* to 34 for *D. episcopa*. The number of polymorphic microsatellites conforming to Hardy–Weinberg expectations (following Bonferroni correction) ranged from 19 (*D. diaboli*) to 27 (*D. argentosa*). One pairwise comparison of microsatellites (in *D. nigrotaeniata*) deviated significantly from expectations of genotypic equilibrium. The microsatellite markers will be useful for conservation and population-genetic studies of these and other species in genus *Dionda*.

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The genus *Dionda* is comprised of spring and stream inhabiting cyprinid fishes found in arid and semi-tropical regions of the North American southwest (Mayden et al. 1992). Species in the United States include the Manantial roundnose minnow (*D. argentosa*), Devil's River minnow (*D. diaboli*), roundnose minnow (*D. episcopa*), Guadalupe roundnose minnow (*D. nigrotaeniata*), and Nueces roundnose minnow (*D. serena*). The Devil's River minnow is listed as critically imperiled and is both state and federally threatened (US Fish and Wildlife Service 1999; TWAP 2008; Garrett et al. 2002). The Manantial and Nueces roundnose minnows are listed as imperiled, while the conservation status of the Guadalupe roundnose minnow is unknown (TWAP 2008). Factors impacting these species include present and future potential threats from habitat degradation, introduced species, and decreased water quality and quantity (Edwards et al. 2004; TWAP 2008; G. Garrett pers. comm.). Herein, we describe development of microsatellite markers that can be used in conservation studies of these and other species of genus *Dionda*. Nuclear-encoded microsatellites can provide valuable genetic information for conservation efforts by assisting in defining conservation units and in the design of captive breeding programs to 'enhance' wild stocks (Saillant et al. 2004, 2005; Turner et al. 2004).

The protocol used to generate the enriched microsatellite library from genomic DNA of *D. episcopa* can be found in Renshaw et al. (2008). Briefly, whole genomic DNA was extracted from muscle tissue of a single individual, using a DNeasy Blood and Tissue Kit (Qiagen); two separate digestions were performed with *Hae*III and *Alu*I (New England BioLabs). Genomic DNA fragments

from both digestions were combined together and adaptors attached with T4 DNA Ligase (Promega). Fragments ranging in size from 500 to 1,500 bp were excised from a 2% agarose gel and cleaned with a QIAquick Gel Extraction Kit (Qiagen); the success of the linker ligation was verified with PCR. The size selected genomic DNA (with linkers ligated) was hybridized in separate reactions with two 3′-biotin-modified oligonucleotides: $(GA)_{13}$ and $(CA)_{13}$, both at 43°C. It is important to note that the PCR products were not used for the hybridization. A significant number of 'chimeric' clones are often generated with this protocol (Renshaw et al. 2008); the impact of 'chimeric' clones can be reduced significantly by using size-selected genomic DNA (with ligated linkers) for hybridizations rather than amplified PCR products.

Each hybridization mix was incubated with streptavidin-coated magnetic M-280 Dynabeads (Invitrogen) for 30 min at ambient temperature and rinsed according to Renshaw et al. (2008). Microsatellite-containing DNA fragments were eluted at 98°C with 100 µl of PCR-grade water. The quantity of enriched DNA was increased with PCR and amplified products were cleaned with QIAquick PCR Purification Kits (Qiagen). Cleaned PCR products were ligated into pGEM-T vector (Promega), using T4 DNA Ligase (Promega), and transformed into *Escherichia coli* (JM109 High Efficiency Competent Cells, Promega). Transformed cells were plated onto X-Gal/IPTG Luria–Bertani (LB) agar with 100 µg/ml of ampicillin and grown overnight at 37°C. Positive (white) colonies were picked with sterile toothpicks, placed in 96-well tissue culture plates with LB broth (containing 100 µg/ml of ampicillin), and incubated at 37°C overnight to increase density of the cultures. Clones were PCR screened for microsatellites per Renshaw et al. (2008).

Colonies identified as positive by PCR-screening were used to inoculate 1 ml cultures of LB broth selective media (ampicillin) and incubated overnight at 37°C. Plasmid DNA was isolated (alkaline lysis) with a BioRobot 8000 (Qiagen). Miniprep DNA was quantified, normalized, and both strands sequenced, using M13 forward and reverse sequencing primers and ABI BigDye Terminator v3.1. Products were amplified and electrophoresed on an ABI 3100 DNA Analyzer (Applied Biosystems). Phred (CodonCode) was used for DNA sequence base calling and vector trimming. A total of 108 PCR primer pairs flanking dinucleotide, trinucleotide, and tetranucleotide microsatellite arrays were designed using Primer3 (http://frodo.wi.mit.edu/).

Unlabelled PCR primers for all 108 primer pairs were purchased from Integrated DNA Technologies (IDT) and tested for amplification by screening three individuals of each of the five species of *Dionda*. Information on collection localities for the five species is below. PCR amplifications were performed with a PTC-200 thermocycler (MJ Research) in 10 µl reaction volumes containing 100 ng DNA, 1× PCR buffer, 0.5 U *Taq* DNA polymerase (GoTaq Flexi DNA Polymerase, Promega), 0.5 µM of each primer, 200 µM of each dNTP, and 2 mM $MgCl_2$. PCR conditions consisted of an initial denaturation at 95°C for 3 min, followed by 38 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 45 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. PCR products were electrophoresed on a 2% agarose gel, stained with ethidium bromide, and visualized under UV light. For primer pairs that appeared to produce amplifications for at least four of the five species (total of 38), one primer from each pair was labeled with one fluorescent label of Set D (Applied Biosystems): 6-Fam or Hex. Characteristics of the 38 primer pairs are summarized in Table 1.

DNA was extracted from 25 individuals of each species; collection localities were the Devil's River in Texas (*D. argentosa* and *D. diaboli*), El Rito Creek (Pecos River drainage) in New Mexico (*D. episcopa*), the Guadalupe River in Texas (*D. nigrotaeniata*), and the Nueces River in Texas (*D. serena*). PCR products were run on an ABI 377 automated sequencer. Alleles were sized using the Genescan®-400 HD Rox Size Standard (Applied Biosystems); allele sizing and calling were performed using Genescan® 3.1.2 and Genotyper® version 2.5 software. Genetic variability of the microsatellite markers was measured by the number of alleles, gene diversity (expected heterozygosity), and observed heterozygosity. Fisher's exact test, as implemented in GDA (Lewis and Zaykin 2001), was used to test significance of departure from Hardy–Weinberg equilibrium (genotype) expectations at each microsatellite and of departure from genotypic equilibrium at pairs of microsatellites. Evidence for occurrences of null alleles was explored using Microchecker (Van Oosterhout et al. 2004).

Summary data are presented in Table 2. The number of microsatellite primers producing experimentally tractable amplifications were as follows: 29 microsatellites (28 polymorphic) in *D. argentosa*; 28 microsatellites (22 polymorphic) in *D. diaboli*; 34 microsatellites (23 polymorphic) in *D. episcopa*; 33 microsatellites (21 polymorphic) in *D. nigrotaeniata*; and 30 microsatellites (28 polymorphic) in *D. serena*. For *D. argentosa*, the number of alleles detected per polymorphic microsatellite ranged from 2 (*Dep* 21, *Dep* 65, and *Dep* 100) to 20 (*Dep* 32); expected heterozygosity ranged from 0.078 (*Dep* 21) to 0.944 (*Dep* 32), while observed heterozygosity ranged from 0.080 (*Dep* 21) to 0.960 (*Dep* 73); genotypes at one microsatellite (*Dep* 101) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). Microchecker identified possible null alleles at three markers (*Dep* 38, *Dep* 91, and *Dep* 101). For *D. diaboli*, the number of alleles

Table 1 Characteristics for 38 microsatellite loci isolated from the roundnose minnow, *Dionda episcopa*

Msat	GenBank[a]	Primer sequence (5′–3′)[b]	Repeat sequence[c]	T_A^d	Fluorescent[e]	Clone size[f]
Dep 1	FJ442952	**ACGCTGACATTAGTGGTTGG** CATTGACATGCTGTCCTTCC	$(GTT)_6$	55	6-FAM	203
Dep 2	FJ442953	**TATTGGGGTTGTGCATCATT** GATGGAATGGCAGGAAGATT	$(GATA)_7$	55	HEX	226
Dep 3	FJ442954	**ACACGGGTAAAAGAGACGAGT** ATGGCAGATAAAGCCTTCCTA	$(GA)_{14}$	55	6-FAM	336
Dep 7	FJ442955	**TGCACTGAAAGGCAGATTTAC** TGTTCTCAGCCTTTACCTCCT	$(GA)_{21}$	55	6-FAM	266
Dep 8	FJ442956	**TTGACTTGGCAGGGTGATTA** GGCTCTGATGACTGTGAGGA	$(CA)_{10}$	55	6-FAM	126
Dep 9	FJ442957	**AACTGGTCGAGCTCCTGTTT** TACTGACGGATCTGGCTGAG	$(CA)_{10}$	55	HEX	152
Dep 10	FJ442958	**AATATCCTCTGGTTGGGAATG** ACTCGCCTGATGAGAAGAAAT	$(GA)_{20}$	55	HEX	305
Dep 12	FJ442959	**CAGCCCATTCTGCATCTAAA** TCCAGTGACATGAAGGGTTT	$(CA)_{12}$	55	HEX	229
Dep 13	FJ442960	**AGCCTTTATCTCCTCCAGTGA** TGTGTGAATAATGTGGTGTGTG	$(GA)_{18}$	52	6-FAM	259
Dep 18	FJ442961	**CCGTGCAGCATTCAAGTAGT** AACAAGGCGGTTCATTGTTT	$(GA)_7AA(GA)_{13}$	55	HEX	143
Dep 20	FJ442962	**TTGCATGCCTGTTCTCTCTC** CCTCAATGCAGGAAGATGAG	$(CA)_{10}CG(CA)_4CG(CA)_6$	55	HEX	200
Dep 21	FJ442963	**AAACTCAAACCGGTCCTCAC** GGTCGGTAAAGCGATAGAGC	$(CA)_9$	52	6-FAM	180
Dep 28	FJ442964	**ATTGGTGTAAATGCCCTGAGTT** GCAGCAATGTGAATGTTTGAGT	$(CA)_{14}$	52	6-FAM	298
Dep 30	FJ442965	**GAAGAAACAAAGGGTGAGATGG** GGGAATGAGCACTGGATAAAAC	$(CA)_{19}$	55	HEX	316
Dep 32	FJ442966	**TGGAACAAAGTTGTCTTGCAGT** TGGACAGGTCAGTAAAAGCAGA	$(GA)_{20}$	52	6-FAM	347
Dep 33	FJ442967	**TAATGAACGCATCAGCAAGACT** ACGCGTTTCTGTTATCTTGTCA	$(CA)_9$	55	HEX	231
Dep 38	FJ442968	**ACACTCTGTCTGGAAGAGCACA** CGATGTCATTGTGGCTAAGTGT	$(CA)_9$	52	HEX	186
Dep 40	FJ442969	**TCATTAGGGCAAAATCCACTGT** GAGAAGTTCCTGAGACCACACC	$(GA)_{14}$	55	6-FAM	251
Dep 44	FJ442970	**TGAGTCGGGCTGAGAGGAT** GTGGAGGAGTGTTTGGGATTTA	$(GA)_{11}$	55	HEX	80
Dep 51	FJ442971	**GCACAAAATTGAGTTTGCATGT** TGGTCCTGTGTTTCTGTCAATC	$(CA)_{10}$	52	HEX	224
Dep 53	FJ442972	**AGTGAGGGAGGAAAGAAAAAGG** GTGCGAAAGAGATTTCCACTTC	$(GA)_{11}$	55	6-FAM	211
Dep 57	FJ442973	**ATTTCCCGCGAAACTAGTAACA** GACCACTTAGCCTGCTCAAATC	$(CA)_{20}$	55	6-FAM	279
Dep 61	FJ442974	**GAAAGGCTTTTTGTCAGTTGCT** CAATACTTTGTGTGCGTTTGTG	$(CA)_{11}$	52	HEX	201
Dep 65	FJ442975	**AAGGTTTGACAGGGAAAAGACA** TGTGTTAAATGAACATAAGACAATCA	$(GA)_{14}$	52	HEX	226

Table 1 continued

Msat	GenBank[a]	Primer sequence (5′–3′)[b]	Repeat sequence[c]	T_A^d	Fluorescent[e]	Clone size[f]
Dep 67	FJ442976	**CATGACGGTTATGAATTCTCCA** GAAAAGTGTGTTTGCACGTGTT	$(CA)_{12}$	55	6-FAM	237
Dep 73	FJ442977	**ATGTGCCCGAACAATAGGAA** TACCAGGGGAGACATAAACAGG	$(CA)_{11}$	55	HEX	176
Dep 74	FJ442978	**CGCCGTTATATTTAGCATCTCC** CGTGCCTGACATAGACATTCTG	$(CA)_{10}$	55	HEX	282
Dep 85	FJ442979	**CCTCAACGCTGACGATATACAC** AGATGCATGTCAAACTCACCAC	$(CA)_9$	55	6-FAM	228
Dep 90	FJ442980	**AAGTCCTACCTGGCTGTTTGAA** TCTTTGAATTACGGTTCCCTGT	$(GA)_{17}$	55	6-FAM	233
Dep 91	FJ442981	**CTCCAATACCGTTGACCTTCTC** GTGGTGACATGACACACAAACA	$(GA)_{16}$	55	HEX	193
Dep 93	FJ442982	**TTGTCTCCCTGGTTTTGTTTTT** ATTATGCAGCATGGCGAAA	$(CA)_{17}$	55	6-FAM	143
Dep 100	FJ442983	**GTTTGGTCCAGAAGGAGTCATC** GGAGGCTTGTGTATTTGTGTGA	$(CA)_{21}$	55	6-FAM	288
Dep 101	FJ442984	**GTCTGTTGGATCTCAGGAGAGG** CTGTCTGTTTTCCATCATCCAA	$(CA)_6CG(CA)_{13}$	52	HEX	192
Dep 102	FJ442985	**CAAGGAGGCATTAAATTGACAAA** TACCCTATCAATATGGGCCAAC	$(TA)_5(CA)_7(TA)_9$	55	6-FAM	267
Dep 103	FJ442986	**GGGAAAACAGACGGTTTATCAT** TACATTATCATTTGCGCCACTC	$(CA)_9$	52	6-FAM	244
Dep 105	FJ442987	**GGAAAACACTGATGAGAGGAGAG** GACGCATAGTGTTCCTGAATGA	$(CA)_4GA(CA)_{11}$	55	HEX	173
Dep 106	FJ442988	**AGCAAAAGCACTCTCACATTCA** TCACAACCACACACACGTTAAA	$(CA)_{10}CCCACG\ (TA)_6$	55	HEX	223
Dep 108	FJ442989	**CACACAAACACGCACTCTGA** TACTCCAATGAAATGCAGCAAG	$(CA)_{14}AA(CA)_5$	52	6-FAM	279

[a] GenBank accession numbers for clone sequences

[b] Primer sequences are forward (top) and reverse (bottom)

[c] Repeat sequence indicates repeat motif

[d] T_A is annealing temperature in °C

[e] Fluorescent indicates the fluorescent label

[f] Clone size is the size (in base pairs) of the clone

The fluorescently labeled primer is in bold

detected per polymorphic microsatellite ranged from 2 (six microsatellites) to 19 (*Dep* 18); expected heterozygosity ranged from 0.078 (*Dep* 53 and *Dep* 106) to 0.950 (*Dep* 90), while observed heterozygosity ranged from 0.080 (*Dep* 53 and *Dep* 106) to 0.960 (*Dep* 3); genotypes at three microsatellites (*Dep* 61, *Dep* 90, and *Dep* 91) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). MICROCHECKER identified possible null alleles at three markers (*Dep* 32, *Dep* 61, and *Dep* 90). For *D. episcopa*, the number of alleles detected per polymorphic microsatellite ranged from 2 (nine microsatellites) to 6 (*Dep* 40); expected heterozygosity ranged from 0.040 (*Dep* 93) to 0.726 (*Dep* 40), while observed heterozygosity ranged from 0.040 (*Dep* 93) to 0.720 (*Dep* 91); genotypes at one microsatellite (*Dep* 91) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). MICROCHECKER did not identify possible null alleles at any of the microsatellites. For *D. nigrotaeniata*, the number of alleles detected per polymorphic microsatellite ranged from 2 (11 microsatellites) to 9 (*Dep* 3); expected heterozygosity ranged from 0.040 (*Dep* 101 and *Dep* 105) to 0.794 (*Dep* 20), while observed heterozygosity ranged from 0.040 (*Dep* 101 and *Dep* 105) to 0.842 (*Dep* 20); genotypes at all microsatellites conformed to Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). MICROCHECKER identified

Table 2 Variability of 38 microsatellites in *D. argentosa* (*Dar*), *D. diaboli* (*Ddi*), *D. episcopa* (*Dep*), *D. nigrotaeniata* (*Dni*), and *D. serena* (*Dse*)

Msat	Species	N^a	N_A^b	Size range[c]	H_E^d	H_O^e	P_{HW}^f
Dep 1	*Dar*	25	3	196–202	0.153	0.160	1.000
	Dep	25	1	202	0.000	0.000	na
	Dni	25	2	202–208	0.184	0.200	1.000
	Dse	25	1	199	0.000	0.000	na
Dep 2	*Dar*	24	9	226–270	0.374	0.417	1.000
	Ddi	24	1	228	0.000	0.000	na
	Dep	24	1	222	0.000	0.000	na
	Dni	22	1	230	0.000	0.000	na
	Dse	na	na	na	na	na	na
Dep 3	*Dar*	na	na	na	na	na	na
	Ddi	25	17	326–374	0.922	0.960	0.722
	Dep	24	2	344–350	0.156	0.167	1.000
	Dni	24	9	333–361	0.765	0.583	0.011*
	Dse	24	17	317–377	0.910	0.875	0.681
Dep 7	*Dar*	25	11	240–270	0.775	0.720	0.287
	Ddi	25	8	265–287	0.789	0.720	0.089
	Dep	25	1	242	0.000	0.000	na
	Dni	na	na	na	na	na	na
	Dse	25	12	250–274	0.869	0.840	0.024
Dep 8	*Dar*	na	na	na	na	na	na
	Ddi	25	1	116	0.000	0.000	na
	Dep	25	3	124–130	0.381	0.320	0.657
	Dni	na	na	na	na	na	na
	Dse	25	2	113–115	0.040	0.040	1.000
Dep 9	*Dar*	25	8	147–161	0.543	0.600	0.482
	Ddi	na	na	na	na	na	na
	Dep	25	1	153	0.000	0.000	na
	Dni	na	na	na	na	na	na
	Dse	25	7	147–163	0.771	0.760	0.453
Dep 10	*Dar*	23	8	280–296	0.852	0.956	0.235
	Ddi	24	1	297	0.000	0.000	na
	Dep	24	2	292–294	0.223	0.167	0.298
	Dni	25	1	288	0.000	0.000	na
	Dse	24	7	288–300	0.712	0.500	0.034*
Dep 12	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	na	na	na	na	na	na
	Dni	na	na	na	na	na	na
	Dse	25	3	209–217	0.079	0.080	1.000
Dep 13	*Dar*	25	11	248–300	0.873	0.840	0.407
	Ddi	25	4	238–246	0.404	0.480	1.000
	Dep	na	na	na	na	na	na
	Dni	25	1	262	0.000	0.000	na
	Dse	na	na	na	na	na	na
Dep 18	*Dar*	na	na	na	na	na	na
	Ddi	25	19	136–180	0.946	0.920	0.309
	Dep	25	2	151–153	0.326	0.400	0.537
	Dni	24	2	132–142	0.223	0.250	1.000
	Dse	21	19	143–233	0.943	0.857	0.161
Dep 20	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	25	3	181–189	0.422	0.400	1.000
	Dni	19	7	181–213	0.794	0.842	0.272
	Dse	na	na	na	na	na	na
Dep 21	*Dar*	25	2	160–162	0.078	0.080	1.000
	Ddi	25	4	187–193	0.724	0.720	0.819
	Dep	25	1	182	0.000	0.000	na
	Dni	24	2	162–188	0.042	0.042	1.000
	Dse	24	18	187–229	0.903	0.583	0.000*
Dep 28	*Dar*	25	6	262–282	0.7406	0.640	0.425
	Ddi	na	na	na	na	na	na
	Dep	25	3	280–290	0.467	0.480	1.000
	Dni	25	2	287–289	0.274	0.240	0.478
	Dse	na	na	na	na	na	na
Dep 30	*Dar*	25	1	277	0.000	0.000	na
	Ddi	25	2	277–279	0.444	0.240	0.029
	Dep	25	1	289	0.000	0.000	na
	Dni	25	3	306–324	0.541	0.680	0.235
	Dse	24	12	290–342	0.800	0.833	0.478
Dep 32	*Dar*	25	20	323–391	0.944	0.920	0.610
	Ddi	25	13	342–390	0.776	0.600	0.027*
	Dep	25	4	325–331	0.154	0.160	1.000
	Dni	25	4	333–353	0.695	0.800	0.695
	Dse	25	18	333–387	0.918	0.680	0.006*
Dep 33	*Dar*	25	7	232–246	0.734	0.840	0.916
	Ddi	25	1	232	0.000	0.000	na
	Dep	25	1	231	0.000	0.000	na
	Dni	25	1	233	0.000	0.000	na
	Dse	25	13	239–299	0.802	0.760	0.461
Dep 38	*Dar*	25	14	172–208	0.889	0.680	0.005*
	Ddi	25	2	182–184	0.429	0.360	0.613
	Dep	25	2	185–195	0.246	0.280	1.000
	Dni	25	1	184	0.000	0.000	na
	Dse	25	1	177	0.000	0.000	na
Dep 40	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	25	6	274–330	0.726	0.600	0.424
	Dni	25	2	260–280	0.301	0.280	1.000
	Dse	25	24	244–364	0.946	0.800	0.001*

Table 2 continued

Msat	Species	N^a	N_A^b	Size range[c]	H_E^d	H_O^e	P_{HW}^f
Dep 44	*Dar*	23	11	71–93	0.870	0.913	0.052
	Ddi	25	14	83–117	0.773	0.800	0.363
	Dep	24	3	87–93	0.528	0.542	1.000
	Dni	23	3	97–107	0.550	0.609	0.511
	Dse	24	8	89–111	0.844	0.917	0.507
Dep 51	*Dar*	25	10	221–247	0.813	0.840	0.419
	Ddi	25	3	239–255	0.220	0.240	1.000
	Dep	23	1	233	0.000	0.000	na
	Dni	25	4	241–253	0.290	0.200	0.019
	Dse	25	7	223–243	0.445	0.520	1.000
Dep 53	*Dar*	25	7	198–210	0.789	0.840	0.319
	Ddi	25	2	214–218	0.078	0.080	1.000
	Dep	25	1	201	0.000	0.000	na
	Dni	24	4	208–224	0.264	0.208	0.385
	Dse	25	8	204–228	0.842	0.760	0.047
Dep 57	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	24	3	287–293	0.462	0.542	0.813
	Dni	25	1	197	0.000	0.000	na
	Dse	na	na	na	na	na	na
Dep 61	*Dar*	25	4	174–204	0.575	0.480	0.006
	Ddi	25	10	173–201	0.724	0.520	0.001*
	Dep	25	2	201–207	0.503	0.480	1.000
	Dni	25	1	193	0.000	0.000	na
	Dse	24	10	145–211	0.644	0.708	0.498
Dep 65	*Dar*	25	2	225–227	0.326	0.320	1.000
	Ddi	na	na	na	na	na	na
	Dep	25	1	245	0.000	0.000	na
	Dni	24	1	219	0.000	0.000	na
	Dse	na	na	na	na	na	na
Dep 67	*Dar*	24	8	224–272	0.731	0.833	0.937
	Ddi	24	2	244–246	0.120	0.125	1.000
	Dep	25	4	237–271	0.614	0.560	0.315
	Dni	25	1	207	0.000	0.000	na
	Dse	25	12	235–279	0.826	0.480	0.001*
Dep 73	*Dar*	25	11	168–194	0.866	0.9600	0.711
	Ddi	25	17	171–235	0.931	0.8800	0.598
	Dep	25	2	170–180	0.184	0.2000	1.000
	Dni	25	1	166	0.000	0.0000	na
	Dse	25	3	168–174	0.189	0.2000	1.000
Dep 74	*Dar*	23	11	280–306	0.839	0.739	0.393
	Ddi	23	9	280–300	0.823	0.783	0.721
	Dep	24	2	284–286	0.191	0.208	1.000
	Dni	25	3	280–298	0.541	0.680	0.2407
	Dse	23	17	291–351	0.944	0.304	0.000*

Table 2 continued

Msat	Species	N^a	N_A^b	Size range[c]	H_E^d	H_O^e	P_{HW}^f
Dep 85	*Dar*	25	6	219–231	0.706	0.760	0.474
	Ddi	25	2	219–223	0.350	0.360	1.000
	Dep	24	3	215–227	0.577	0.417	0.252
	Dni	25	2	223–225	0.411	0.400	1.000
	Dse	24	10	217–245	0.855	0.958	0.931
Dep 90	*Dar*	25	6	218–234	0.766	0.800	0.908
	Ddi	25	18	235–277	0.950	0.720	0.000*
	Dep	25	3	232–242	0.535	0.600	0.497
	Dni	25	4	239–261	0.665	0.600	0.492
	Dse	25	8	212–228	0.664	0.600	0.052
Dep 91	*Dar*	25	17	188–224	0.932	0.760	0.029*
	Ddi	25	14	194–228	0.913	0.800	0.000
	Dep	25	3	196–200	0.545	0.720	0.003
	Dni	25	1	169	0.000	0.000	na
	Dse	25	20	182–230	0.946	0.880	0.201
Dep 93	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	25	2	140–144	0.040	0.040	1.000
	Dni	25	3	152–176	0.358	0.440	0.612
	Dse	na	na	na	na	na	na
Dep 100	*Dar*	25	2	260–264	0.274	0.240	0.493
	Ddi	25	2	262–264	0.184	0.200	1.000
	Dep	25	2	266–268	0.078	0.080	1.000
	Dni	25	1	258	0.000	0.000	na
	Dse	25	3	252–258	0.548	0.760	0.040
Dep 101	*Dar*	25	8	189–209	0.806	0.400	0.000*
	Ddi	25	5	179–187	0.520	0.640	0.702
	Dep	na	na	na	na	na	na
	Dni	25	2	213–223	0.040	0.040	1.000
	Dse	25	8	168–198	0.657	0.720	0.520
Dep 102	*Dar*	na	na	na	na	na	na
	Ddi	na	na	na	na	na	na
	Dep	na	na	na	na	na	na
	Dni	25	2	271–273	0.274	0.240	0.495
	Dse	na	na	na	na	na	na
Dep 103	*Dar*	25	7	250–264	0.724	0.800	0.680
	Ddi	25	3	244–252	0.308	0.360	1.000
	Dep	25	4	268–276	0.535	0.560	0.054
	Dni	25	2	248–252	0.481	0.440	0.696
	Dse	25	4	238–244	0.734	0.680	0.564
Dep 105	*Dar*	25	6	152–166	0.326	0.360	1.000
	Ddi	25	1	171	0.000	0.000	na
	Dep	25	1	166	0.000	0.000	na
	Dni	25	2	171–175	0.040	0.040	1.000
	Dse	25	4	152–162	0.419	0.400	0.665

Table 2 continued

Msat	Species	N^a	N_A^b	Size range[c]	H_E^d	H_O^e	P_{HW}^f
Dep 106	*Dar*	25	6	211–221	0.7478	0.600	0.267
	Ddi	25	2	209–211	0.0784	0.080	1.000
	Dep	25	4	211–223	0.5584	0.600	0.531
	Dni	25	2	209–211	0.4286	0.520	0.388
	Dse	25	4	217–223	0.4857	0.480	0.879
Dep 108	*Dar*	21	5	263–275	0.704	0.667	0.572
	Ddi	na	na	na	na	na	na
	Dep	21	3	277–283	0.473	0.381	0.420
	Dni	na	na	na	na	na	na
	Dse	25	3	215–227	0.431	0.320	0.018

[a] N is the number of individuals assayed

[b] N_A is the number of alleles detected

[c] Size range refers to alleles thus far uncovered

[d] H_E is expected heterozygosity

[e] H_O is observed heterozygosity

[f] P_{HW} represents the probability of deviation from Hardy–Weinberg expectations. Microsatellite/species combinations that failed to produce experimentally tractable amplifications and Hardy–Weinberg probability estimates for monomorphic loci are indicated by 'na'. Probabilities of significant deviations from Hardy–Weinberg equilibrium expectations are underlined; possible occurrences of null alleles, as indicated by MICROCHECKER (Van Oosterhout et al. 2004), are noted by an asterisk (*)

possible null alleles at one marker (*Dep* 3). For *D. serena*, the number of alleles detected per polymorphic microsatellite ranged from 2 (*Dep* 8) to 24 (*Dep* 40); expected heterozygosity ranged from 0.040 (*Dep* 8) to 0.946 (*Dep* 40 and *Dep* 91), while observed heterozygosity ranged from 0.040 (*Dep* 8) to 0.958 (*Dep* 85); genotypes at four microsatellites (*Dep* 21, *Dep* 40, *Dep* 67, and *Dep* 74) deviated significantly from Hardy–Weinberg expectations following Bonferroni correction (Rice 1989). MICROCHECKER identified possible null alleles at six markers (*Dep* 10, *Dep* 21, *Dep* 32, *Dep* 40, *Dep* 67, and *Dep* 74). One pairwise comparison of microsatellites (*Dep* 20/*Dep* 32 in *D. nigrotaeniata*) deviated significantly from genotypic equilibrium ($P = 0.000$), suggesting that these two microsatellites may be linked in this species. The 38 microsatellites developed in this work will prove useful for conservation and population genetic studies in these and other species of genus *Dionda*.

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