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Wildlife Research

Supplementary Material

Comparison of morphological identification and DNA metabarcoding for dietary analysis of faeces from a subtropical lizard

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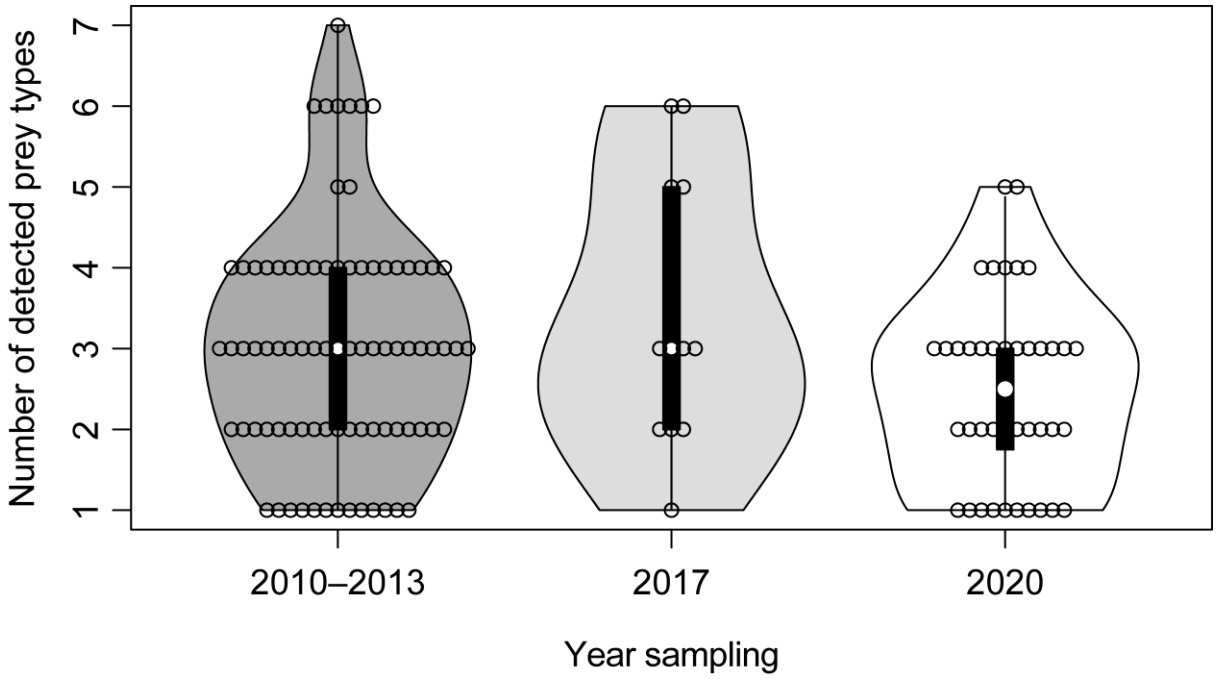

Figure A1. Violin plot for the relationship between sampling years and the number of detected prey types by the faecal DNA metabarcoding analyses. No significant difference in the prey type numbers was observed among the sampling years (see the main text for statistical test), suggesting that DNA degradation in preservation causes little effect to the results of the dietary analyses.

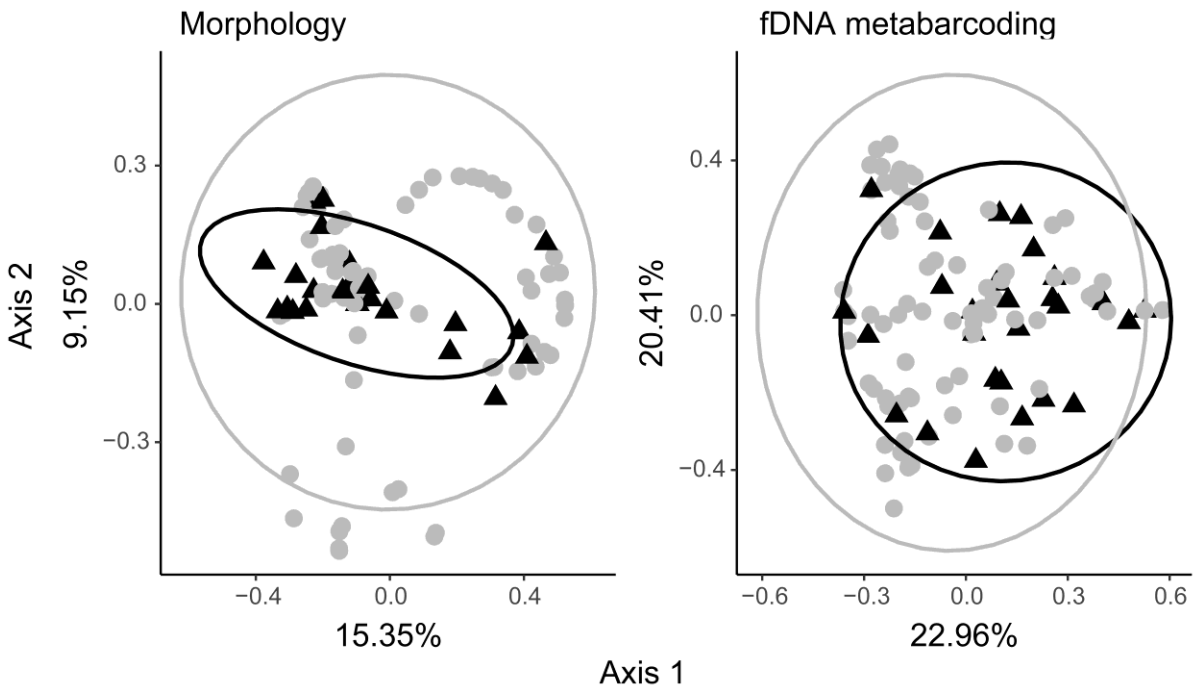

Figure A2. Difference of inferred prey diversity between gecko's size classes (black triangles and an ellipses for juvenile; gray circles and an ellipses for adults) measured by the Bray–Curtis similarity coefficients for the morphology data and the Jaccard coefficients for the fDNA metabarcoding data, and visualised using the principle coordinate analysis (PCoA). The ellipses are the 95% confidence ellipses.

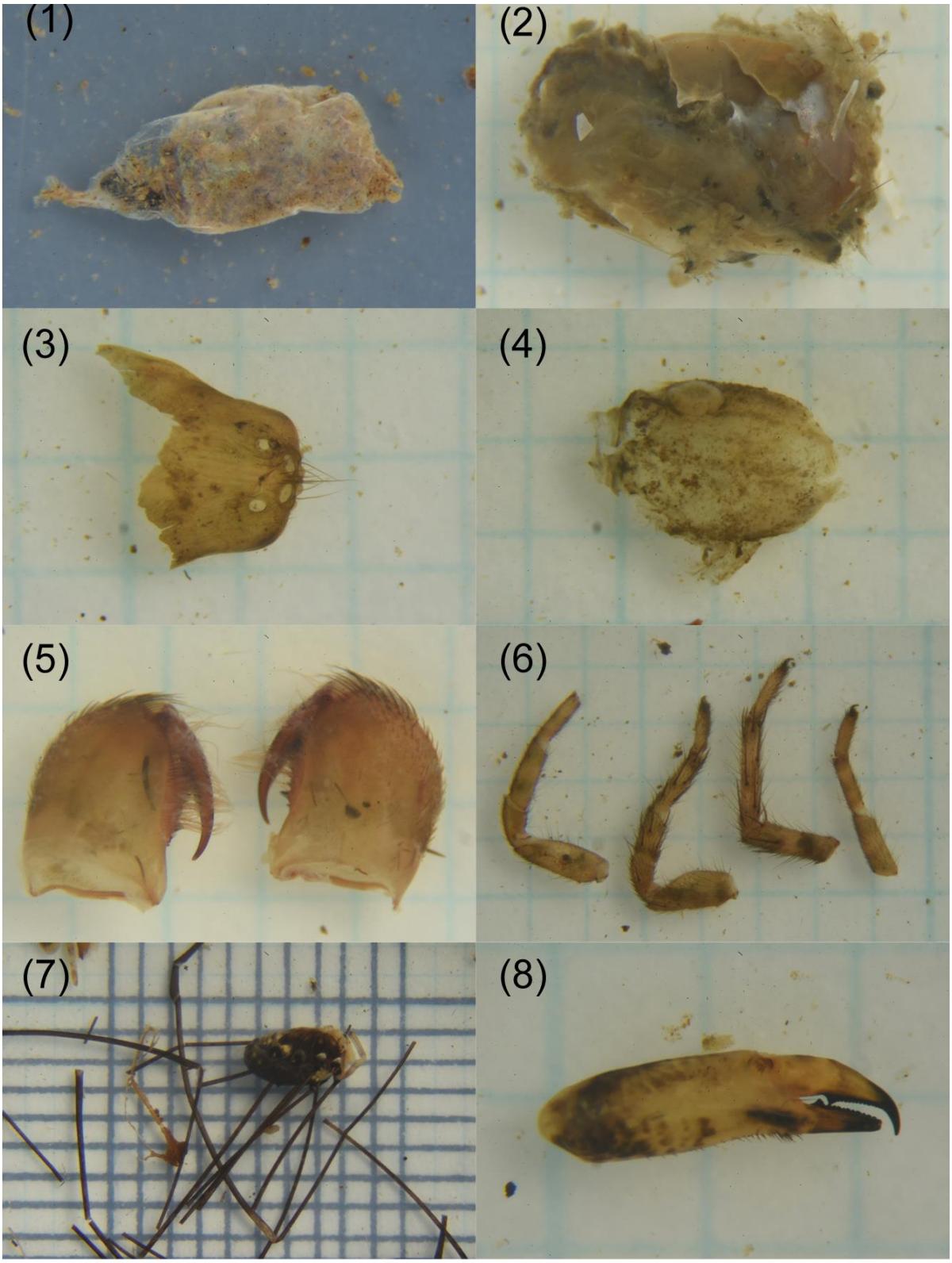

Figure A3. Remains in feces of *Goniurosaurus kuroiwae*. Each square has 1-mm long edges. (1) Possible remain of Crassiclitellata but was not identified morphologically; (2) shell of Stylommatophora; (3) cephalothorax, (4) abdomen, (5) chericerae, and (6) legs of Araneae; (7) whole body and (8) chelicera of Opiliones. (Figure continues)

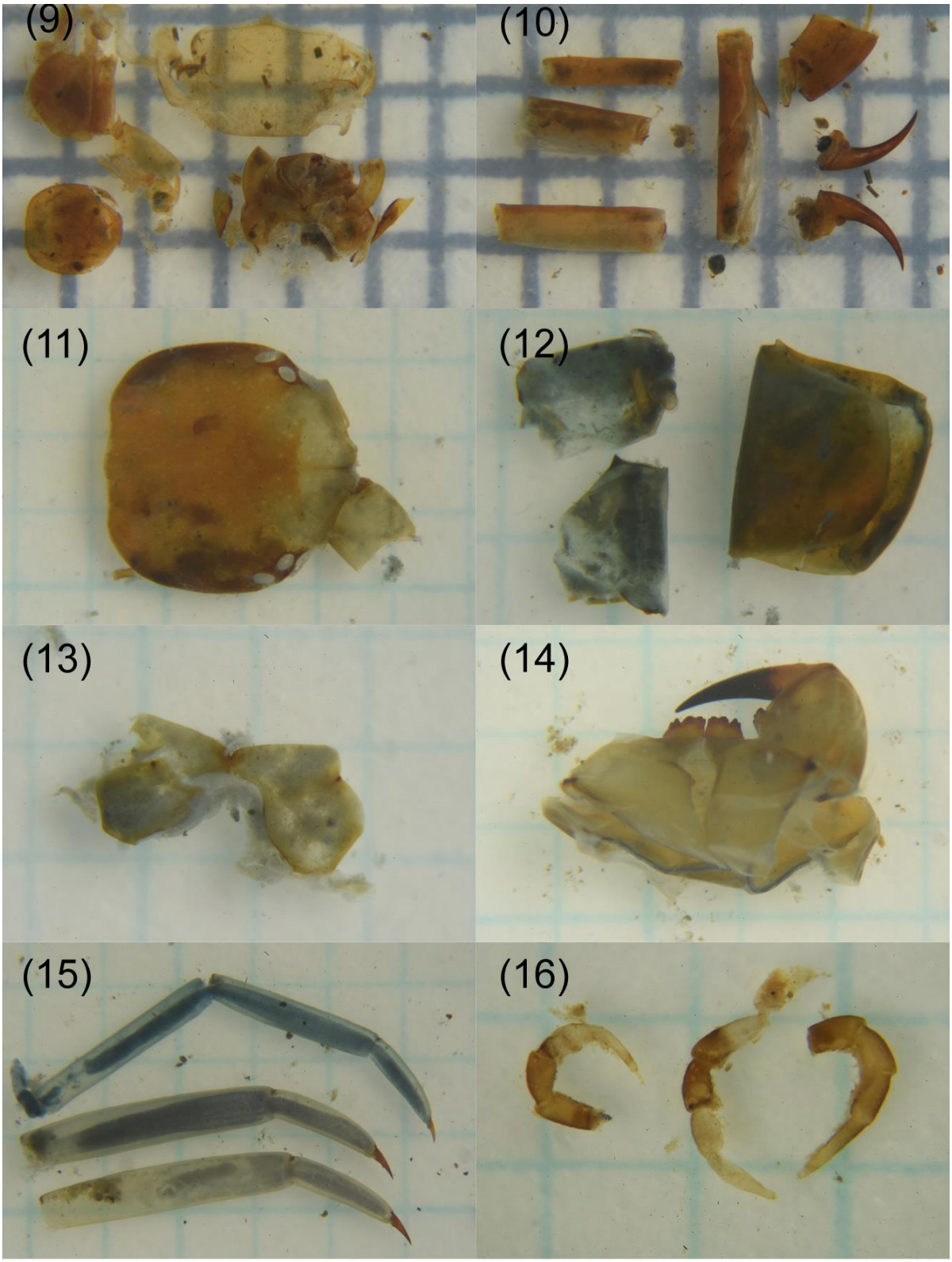

Figure A3. (9) Head capsule, body plates, (10) tarsungulum, and legs of Lithobiomorpha; (11) head capsule, (12) body plates, (13) maxilla, (14) coxosternite, tarsungulum, and (15) legs of Scolopendromorpha; (16) legs of Polydesmida.

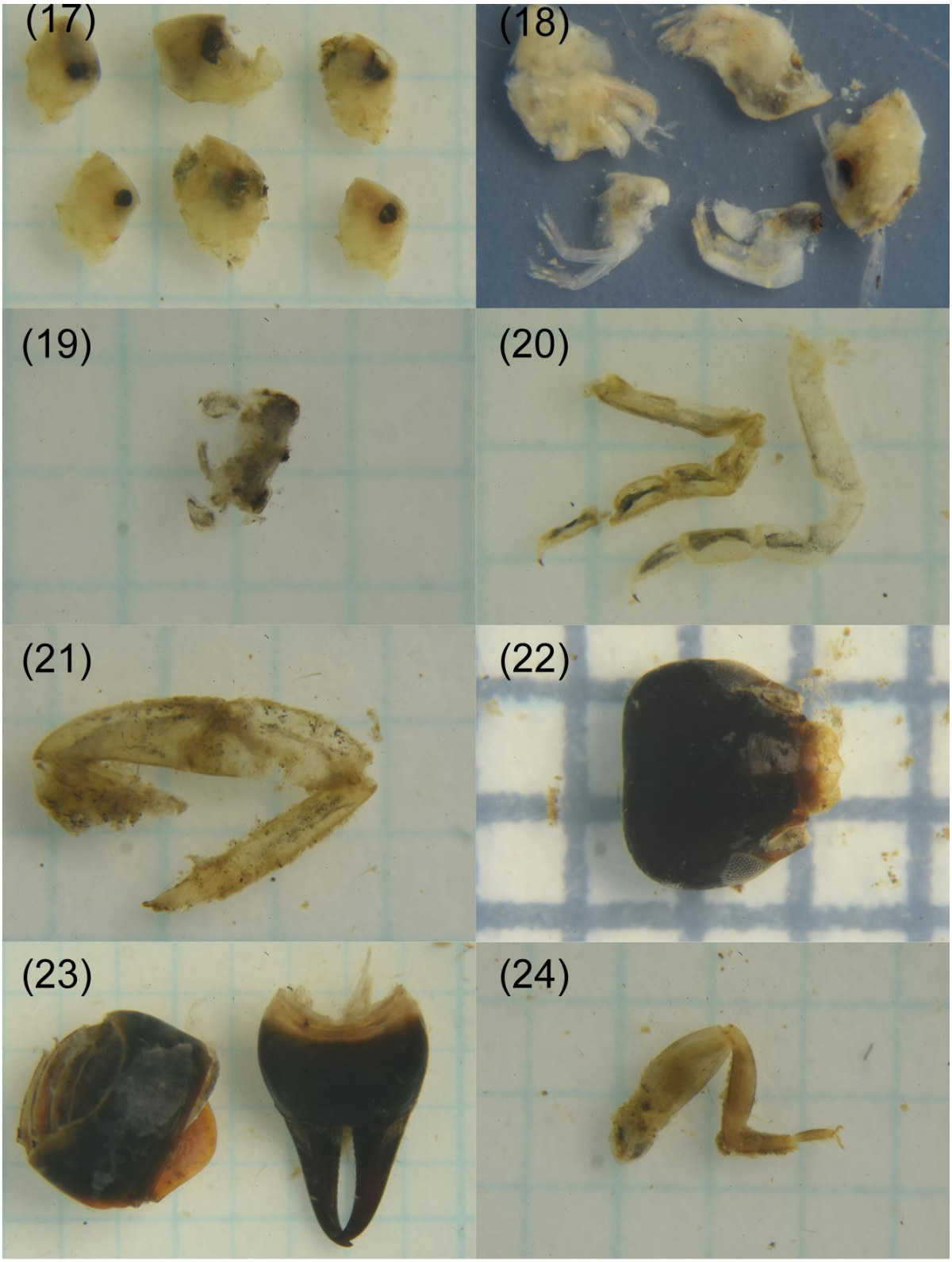

Figure A3. (17) Heads, (18) bodies, and legs of Amphipoda; (19) body plate and (20) legs of Isopoda; (21) leg of Decapoda; (22) head, (23) abdomen, cerci, and (24) leg of Dermaptera.

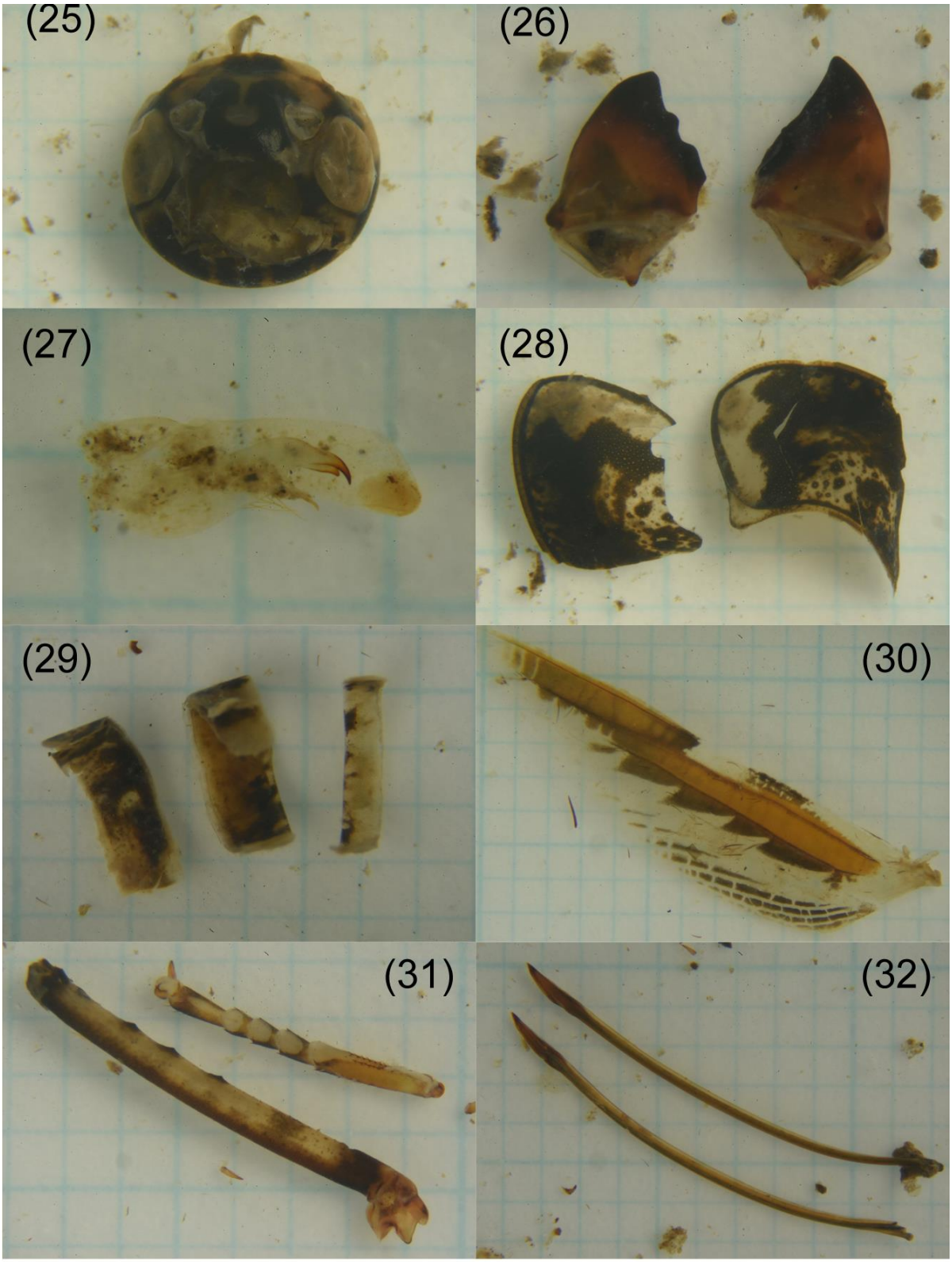

Figure A3. (25) Head, (26) mandibles, (27) maxilla, (28) thorax plates; (29) abdominal plates; (30) wing, (31) legs, and (24) oviducts of Orthoptera.

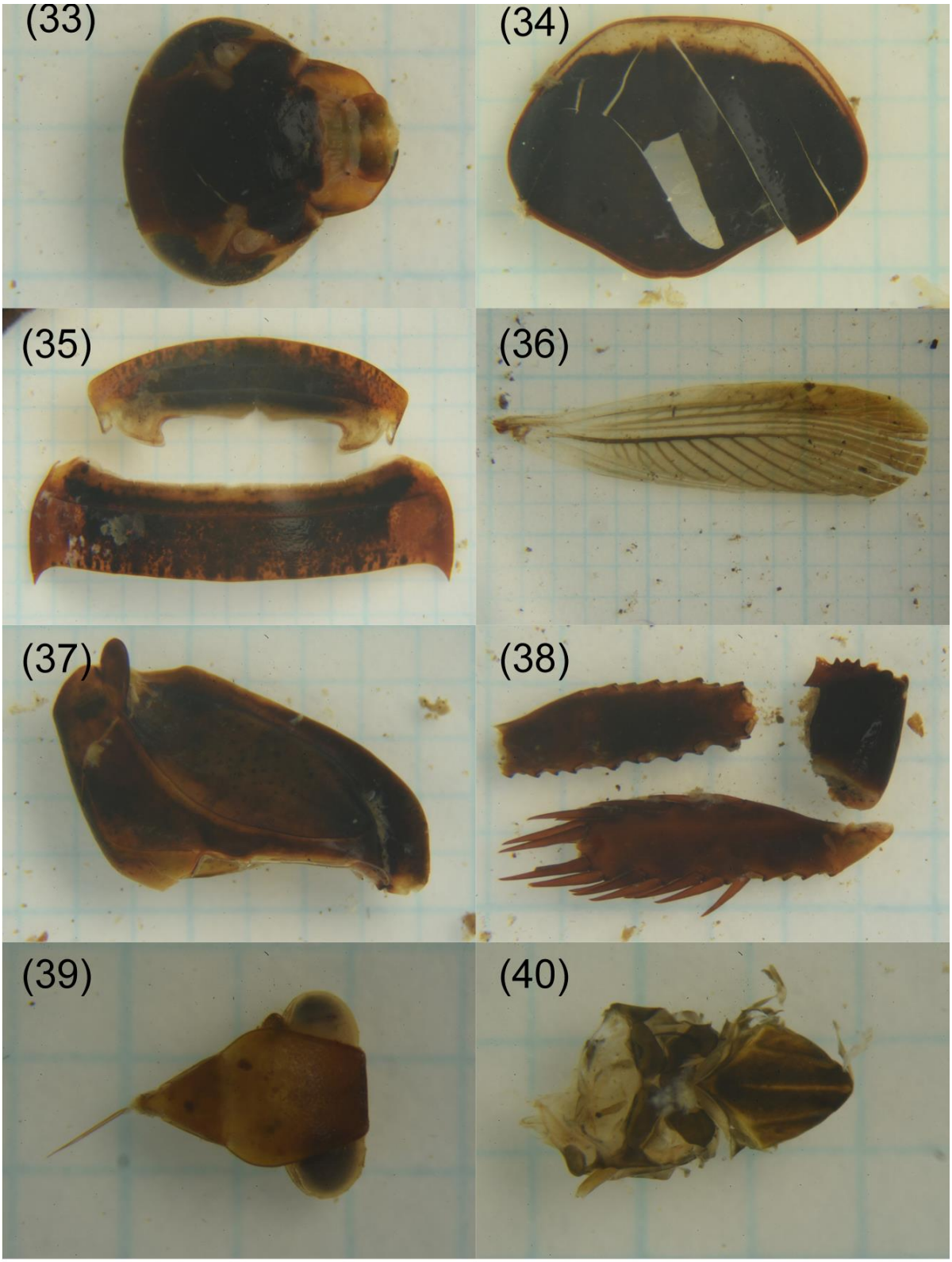

Figure A3. (33) Head, (34) thorax plate, (35) abdominal plates, (36) wing; (37) coxa, and (38) legs of Blattodea; (39) head and (40) body of Hemiptera.

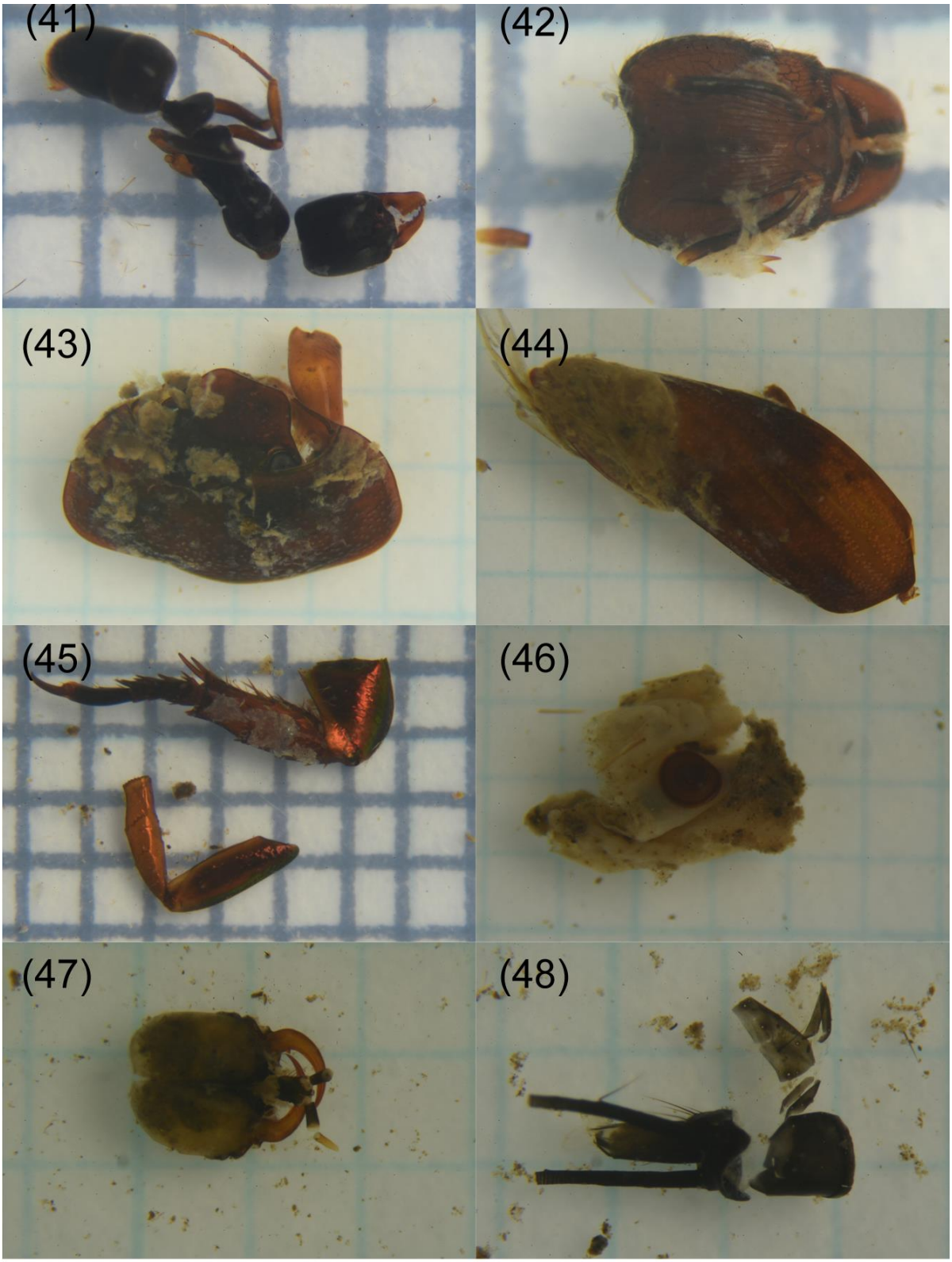

Figure A3. (41) Whole body and (42) head of Hymenoptera; (43) head, (44) forewing, and (45) legs of adults, and (46) skin, (47) head, and (48) cerci of larvae of Coleoptera.

(continued)

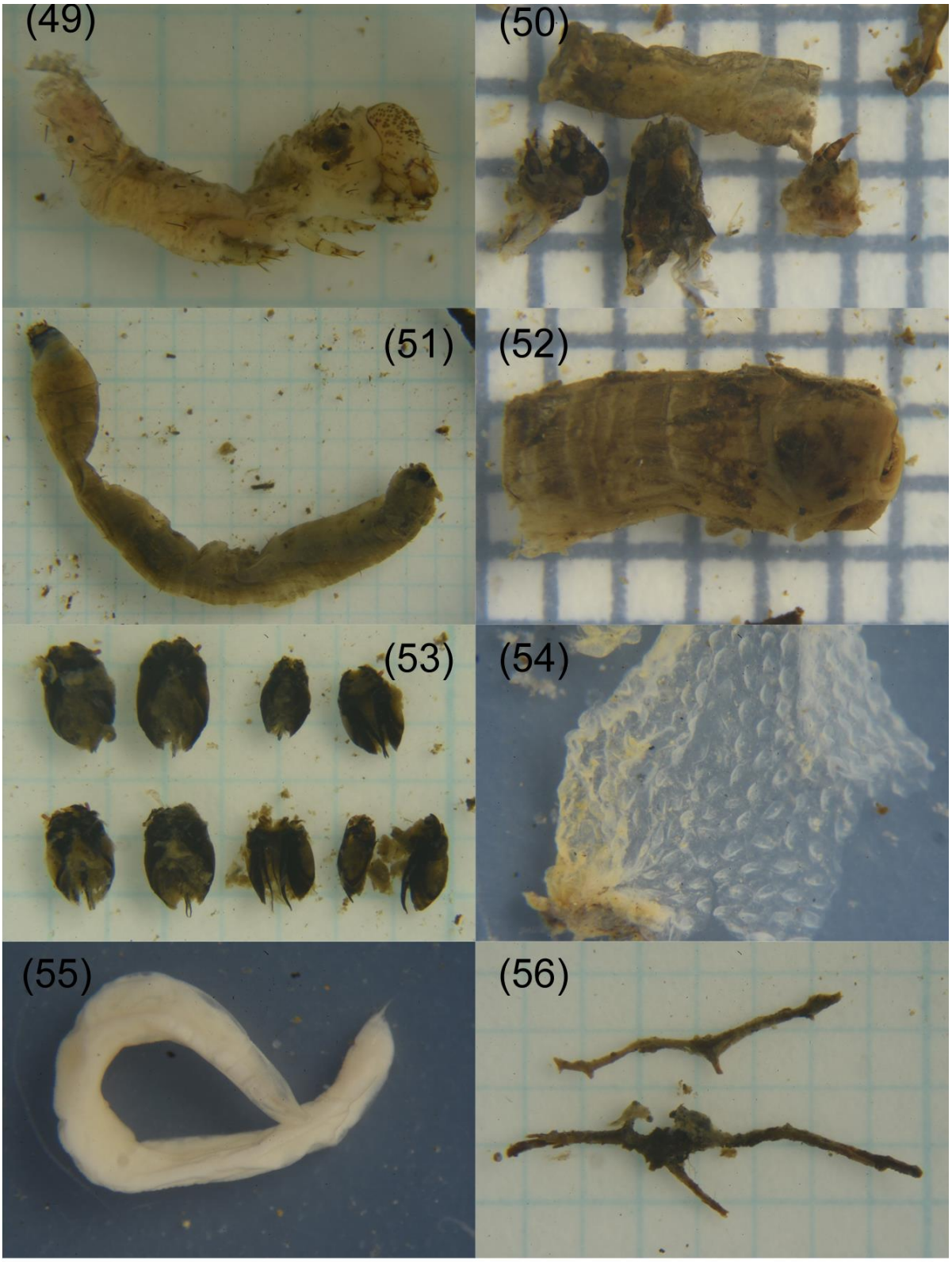

Figure A3. (49, 50) Head and legs of larvae of Lepidoptera; (51) whole body, (52) head, and (53) cephalopharyngeal skeletons of larvae of Diptera. Other non-prey remains such as (54) shed skin of *G. kuroiwae*, (55) internal parasite, and (56) dead plants were also included.

Figure A4. Examples of earthworm predation by *Goniurosaurus* geckos. A naturally regurgitated earthworm, probably due to too big size for this gecko, from *G. kuroiwae* in the northern part of Okinawajima Island (upper) and an earthworm resisting predation by *G. orientalis*, a closely related species with *G. kuroiwae*, in Tonakijima Island (lower).

Table A1. The groups of eukaryotic organisms detected from fecal samples of *G. kuroiwae* by the morphological and genetic identification. "+" indicates present but not counted. In the morphological assay, the estimated minimum number of prey individuals is shown. In the metabarcoding analyses, the number of ASVs detected on the basis of each lous is indicated.

Classification	Undigested materials (n=103)					Metabarcoding ASVs (n=160)			
	Samples contain	Total number	Max per sample	Mean per sample	Min per sample	16S-S	16S-L	COI-S	COI-L
Oomycota*									1
Amoebozoa*								3	2
Ascomycota*						12			24
Basidiomycota*								3	2
Mucoromycota*								7	11
Rotifera*							2		3
Mollusca									
Gastropoda									
Stylommatophora	2	2	1	1.00	1				
Annelida									
Clitellata									
Crassiclitellata						16		27	4
Nematoda*	+								25
Arthropoda									
Arachnida									
Araneae	33	35	2	1.06	1	10	6	7	4
Opiliones	3	3	1	1.00	1	2			
Sarcoptiformes*								1	
Trombidiformes*								1	2
Chilopoda									
Lithobiomorpha	3	3	1	1.00	1	2	1	1	
Scolopendromorpha	15	19	3	1.27	1	23	9	11	8
Diplopoda									
Glomerida							4	2	2
Polydesmida	2	2	1	1.00	1	1		3	4
Malacostraca									
Amphipoda	16	34	6	2.13	1	18	4	9	5
Isopoda	2	2	1	1.00	1	11	2	7	5
Decapoda	1	1	1	1.00	1			1	
Collembola*						4	1	5	
Insecta									
Archaeognatha							1		
Plecoptera						2			
Dermaptera	13	16	3	1.23	1	3	1		
Orthoptera	45	53	3	1.18	1	51	24	24	5
Phasmatodea						4	1		
Blattodea	17	18	2	1.06	1	14	10		5
Hemiptera	2	2	1	1.00	1	5	1	4	
Psocoptera*						3			1
Hymenoptera	2	2	1	1.00	1	8	1	1	2
Coleoptera	4	4	1	1.00	1	16	4	2	1
Lepidoptera	19	27	5	1.42	1	17	9	23	3
Diptera	10	37	11	3.70	1	9	4	10	1
Chordata*	+					1	3		
Total		260				232	88	152	120

* Groups unlike prey of *G. kuroiwae*

Table A2. Prey items identified at species level in the DNA metabarcoding analyses. Maximum body length of the species (mm) were shown on the right.

Species identified	Body length
Annelida	
Clitellata	
Crassiclitellata	
Amynthas corticis	184
Amynthas gracilis	158
Amynthas morrisi	126
Duplodicodrilus schmardae	120
Metaphire californica	156
Pontoscolex corethrurus	128
Arthropoda	
Arachnida	
Araneae	
Heptathela tokashiki *	11
Heteropoda venatoria	30
Pardosa laura	7
Chilopoda	
Scolopendromorpha	
Rhysida immarginata	70
Scolopendra japonica	110
Diplopoda	
Polydesmida	
Chamberlinius hualienensis	30
Oxidus gracilis	20
Malacostraca	
Amphipoda	
Platorchestia japonica	11
Talitroides alluaudi	5.3
Talitroides topitotum	9
Isopoda	
Armadillidium vulgare	18
Burmoniscus dasystylus	10.5
Burmoniscus kathmandius	6.9
Burmoniscus meeusei	7.7
Porcellionides pruinosus	13
Insecta	
Dermaptera	
Euborellia annulipes	25
Euborellia arcanum	26
Orthoptera	
Cardiodactylus guttulus	37
Fer nigripennis *	?
Hexacentrus japonicus	47
Loxoblemmus equestris	13
Mecopoda elongata	75
Melanogryllus bilineatus	16
Ornebius bimaculatus	15
Ornebius kanetataki	11

Table A2. Continued.

Species identified	Body length
Orthoptera	
Polionemobius taprobanensis	7
Teleogryllus occipitalis	31
Traulia minuta *	?
Velarifictorus micado	16
Velarifictorus ornatus	15
Phasmatodea	
Micadina phluctainoides	56
Neohirasea japonica	75
Blattodea	
Asiablatta kyotensis *	18
Lobopterella dimidiatipes	10
Opisthoplatia orientalis	35
Periplaneta australasiae	30
Pycnoscelus indicus	18
Pycnoscelus surinamensis	18
Hemiptera	
Euterpnosia chibensis	29
Yezoterpnosia vacua *	32
Hymenoptera	
Kradibia gibbosae *	?
Pheidole fervens	4.5
Coleoptera	
Brahmina crenicollis *	20
Diplocheila zealandica	26
Lepidoptera	
Ectropis crepuscularia	25
Hiradonta ohashii	50
Hydrillodes lentalis	30
Hydrillodes metisalis *	30
Paralipsa gularis	20
Parnassius jacquemontii *	20
Plusiopalpa adrasta	32
Scopula subpunctaria *	30
Diptera	
Gymnopternus blankaartensis *	?

* Species not recorded from the study area

Table A3. The result of PERMDISP and perMANOVA tests for two methods of prey identification..

	Df	SS	MS	F	η^2 (R^2)	p-value	
PERMDISP							
Methods	1	0.017	0.017	2.267	0.038	0.130	
Residuals	158	0.426	0.004				
perMANOVA							
Methods	1	3.328	3.328	17.578	0.054	< 0.001	*
Individuals	79	43.715	0.553	2.922	0.705	< 0.001	*
Residuals	79	14.958	0.189		0.241		

* Statistically significant at a significance level of $\alpha = 0.05$

Df, degree of freedom; SS, sum of squares; MS, mean square

Table A4. The frequency-based and abundance-based indices of importance of each prey type for each dataset. FO (/103) and FO (/134) indicate the frequency of occurrence to the samples that contained at least one prey item and FO (/177) indicates the frequency of occurrence to all examined samples.

	Morphology				Metabarcoding			Combined	
	FO (/103)	FO (/177)	wPO	IRI	FO (/134)	FO (/177)	wPO	FO	wPO
Mollusca									
Gastropoda									
Stylommatophora	1.9	1.1	1.3	0.1	-	-	-	1.3	0.6
Annelida									
Clitellata									
Crassiclitellata	-	-	-	-	45.5	34.5	17.3	43.8	10.6
Arthropoda									
Arachnida									
Araneae	32.0	18.6	15.6	11.0	12.7	9.6	4.2	37.5	9.2
Opiliones	2.9	1.7	1.1	0.0	1.5	1.1	0.4	3.8	1.1
Chilopoda									
Lithobiomorpha	2.9	1.7	1.8	0.1	0.7	0.6	0.4	-	-
Scolopendromorpha	14.6	8.5	9.4	6.5	14.9	11.3	6.2	20.0	7.1
Diplopoda									
Glomerida	-	-	-	-	4.5	3.4	1.3	2.5	0.8
Polydesmida	1.9	1.1	0.8	0.0	4.5	3.4	1.4	6.3	1.5
Malacostraca									
Amphipoda	15.5	9.0	7.8	0.6	34.3	26.0	13.5	42.5	12.5
Isopoda	1.9	1.1	1.5	0.0	23.9	18.1	8.0	31.3	8.5
Decapoda	1.0	0.6	0.3	0.0	0.7	0.6	0.1	1.3	0.2
Insecta									
Dermaptera	12.6	7.3	5.2	1.8	9.0	6.8	2.5	18.8	4.1
Orthoptera	43.7	25.4	27.7	57.8	39.6	29.9	14.6	46.3	13.4
Phasmatodea	-	-	-	-	3.0	2.3	1.4	2.5	0.5
Blattodea	16.5	9.6	9.3	18.7	15.7	11.9	4.4	25.0	6.5
Hemiptera	1.9	1.1	0.5	0.0	4.5	3.4	1.2	6.3	1.4
Hymenoptera	1.9	1.1	0.4	0.0	16.4	12.4	4.8	18.8	4.1
Coleoptera	3.9	2.3	2.1	0.4	15.7	11.9	4.6	15.0	3.6
Lepidoptera	18.4	10.7	8.9	1.5	33.6	25.4	10.9	41.3	11.2
Diptera	9.7	5.6	6.3	1.5	7.5	5.6	2.7	13.8	3.2

Table A5. The result of PERMDISP and perMANOVA tests for two types of datasets.

	Df	SS	MS	F	η^2 (R^2)	p-value	
Morphology (Bray–Curtis)							
PERMDISP							
Groups	3	0.002	0.001	0.119	0.004	0.946	
Residuals	99	0.426	0.004				
perMANOVA							
Juvenile/Adult	1	0.874	0.874	2.001	0.019	0.012	*
Environment type	1	0.598	0.598	1.368	0.013	0.134	
Residuals	100	43.673	0.437		0.967		
Metabarcoding (Jaccard)							
PERMDISP							
Groups	3	0.112	0.037	4.769	0.099	0.003	*
Residuals	130	1.019	0.008				
perMANOVA							
Juvenile/Adult	1	0.661	0.661	1.808	0.014	0.060	
Environment type	1	0.191	0.191	0.521	0.004	0.883	
Residuals	131	47.900	0.366		0.983		

* Significant at a significance level of $\alpha = 0.05$

Df, degree of freedom; SS, sum of squares; MS, mean square

Table A6. The evaluation of indicator prey types in juvenile and adult geckos by the indecspecies package. For each prey type, a group (J, juvenile; A, adult; and T, total) showing the highest statistic value, which was the square root of the product of specificity (A) and sensitivity (B; FO of the prey type) in juvenile and adult and the square root of FO in the total samples, was indicated.

	Juvenile		Adult				
	A	B	A	B	Statistic		p-value
Morphology (Bray–Curtis)							
Stylommatophora	0.000	0.000	1.000	0.026	0.162	A	0.802
Araneae	0.370	0.370	0.630	0.303	0.566	T	
Opiliones	0.000	0.000	1.000	0.039	0.199	A	0.565
Lithobiomorpha	0.954	0.074	0.046	0.013	0.266	J	0.068
Scolopendromorpha	0.004	0.037	0.996	0.184	0.428	A	0.054
Polydesmida	0.771	0.037	0.229	0.013	0.169	J	0.259
Amphipoda	0.590	0.333	0.410	0.092	0.444	J	0.037 *
Isopoda	0.000	0.000	1.000	0.026	0.162	A	0.807
Decapoda	0.000	0.000	1.000	0.013	0.115	A	1.000
Dermaptera	0.329	0.074	0.671	0.145	0.355	T	
Orthoptera	0.076	0.370	0.924	0.461	0.661	T	
Blattodea	0.313	0.185	0.687	0.158	0.406	T	
Hemiptera	1.000	0.074	0.000	0.000	0.272	J	0.068
Hymenoptera	0.000	0.000	1.000	0.026	0.162	A	0.805
Coleoptera	0.006	0.037	0.994	0.039	0.198	A	0.603
Lepidoptera	0.198	0.148	0.802	0.197	0.429	T	
Diptera	0.152	0.185	0.848	0.066	0.312	T	
Metabarcoding (Jaccard)							
Crassiclitellata	0.530	0.500	0.470	0.443	0.675		
Araneae	0.674	0.214	0.326	0.104	0.380	J	0.125
Opliones	0.791	0.036	0.209	0.009	0.168	J	0.373
Lithobiomorpha	0.000	0.000	1.000	0.009	0.097	A	1.000
Scolopendromorpha	0.558	0.179	0.442	0.142	0.386		
Glomerida	0.000	0.000	1.000	0.057	0.238	A	0.344
Polydesmida	0.654	0.071	0.346	0.038	0.216	J	0.610
Amphipoda	0.689	0.607	0.311	0.274	0.647	J	0.001 **
Isopoda	0.694	0.429	0.306	0.189	0.545	J	0.011 *
Decapoda	0.000	0.000	1.000	0.009	0.097	A	1.000
Dermaptera	0.256	0.036	0.744	0.104	0.299		
Orthoptera	0.498	0.393	0.502	0.396	0.629		
Phasmatodea	0.000	0.000	1.000	0.038	0.194	A	0.575
Blattodea	0.654	0.250	0.346	0.132	0.404	J	0.116
Hemiptera	0.431	0.036	0.569	0.047	0.212		
Hymenoptera	0.602	0.214	0.398	0.142	0.396		
Coleoptera	0.486	0.071	0.514	0.075	0.273		
Lepidoptera	0.486	0.321	0.514	0.340	0.580		
Diptera	0.527	0.179	0.473	0.160	0.405		

Significant at a level of $\alpha = 0.05$ before* and after** the Holm–Bonferroni correction

Supplementary File 1. Primer validation.

Methods

To evaluate prey detection power of the four primer sets employed (16S-short, 16S-long, COI-short, and COI-long), the numbers of prey taxa detected by use of each primer set were counted and compared (Table S1). Amphipoda or larger-sized animals were considered as putative prey (See "Identification of prey in fecal samples" section for the details). The amplicon sequence variants (ASVs) assigned to specific unique references were summed up to each taxonomic id and each id was treated as an independent prey taxon. To assess the amplification specificity, the number of ASVs assigned to non-prey taxa was also counted for each primer set. The ASVs assigned to non-prey taxa were merged at the order level because many putative non-prey taxa, such as amoeba and fungi, were rarely identified to lower taxonomic ranks. Prey detectability and erroneous detections of non-prey organisms are trade-off and highly affected by target length of PCR: shorter fragment would be easily amplified but inferior in accurate taxon identification by BLAST search.

In addition, possible false negative in prey detection was evaluated by in silico PCR implemented in the OBI Tools package (Boyer et al., 2014). Metazoan 16S rRNA sequences were retrieved from the DDBJ/EMBL/GenBank database and their COI sequences were downloaded from the COInr database (Meglécz, 2022). Taxonomic dump files were downloaded from the NCBI Taxonomy database. The sequences unverified by the database curators and those with taxonomic ids yet to be listed in the taxonomy databases were removed by standard computational works. The sequences were annotated to have genus name and taxonomic ids of the genera in the sequence title ("obiannotate" function). To remove the bias in the number of available sequences among taxa, one sequence was randomly selected from a set of sequences that had the same generic taxonomic ids and used as references in the subsequent step ("obiuniq", "obiselect", and "obiconvert" functions). In the in silico PCR ("ecoPCR" function), four mismatches between the reference and entire primer sequences as well as one mismatch in the three base pairs from 3' end of primer sequence were allowed for each primer. The ratio of the number of amplifiable sequences to the number of sequences in the reference were calculated for each primer set ("ecotaxstat" funciton).

The efficiency of suppression of the lizards' DNA amplification by the blocking oligo "blocking-Goni-COI" was checked by PCR using tissue-derived total DNA of an earthworm, a centipede, and two specimens of *G. kuroiwae*. The PCR was conducted in a 9.5 μl solution containing 4 μl of 2× QIAGEN Multiplex PCR Master Mix (Qiagen), 2.5 pmol of each primer set, 5, 25, 50, or 100 pmol of the blocking primer, and 10 ng of total DNA with the following steps: 95°C for 15 min; 35 cycles at 95°C for 30 sec, 40°C for 30 sec, and 72°C for 60 sec; and 70°C for 5 min. The amplification was checked by 3.0% agarose-gel electrophoresis with TAE buffer.

Results

For the efficiency of prey detection, 16S-short and COI-short yielded better scores than the longer ones (Table S2): the numbers of samples for which one or more prey detected by use of 16S-short, 16S-long, COI-short, and COI-long were 153, 58, 131, and 53, respectively. The number of prey types identified to the species level reached to plateau in all primer set, and the numbers of species detected were 47, 17, 36, and 15 in order. Meanwhile, the number of non-prey ASVs were 7, 4, 6, and 43, respectively. The detected prey orders were consistent between the two genes only in 46 out of 160 samples examined. The comparison of sequences between primers and corresponding binding sites were provided in FigureS1.

In the DNA metabarcoding, the primer sets designed for short amplicons had higher detectability of prey species for fecal DNA. One possible shortcoming of an analysis based on shorter sequences is insufficient resolution of species (or higher taxon) identifications, but it was not the case in the present study. Furthermore, high success ratio of amplification in shorter amplicons increases the number of reads and it enables us to discriminate effective reads of given taxon from others. The number of species detected by the short amplicons approach were approximately two to three times larger than the longer amplicon approach (Table S2). The detection of non-prey ASVs were the same level or even worse in the latter approach. Thus, the shorter-amplicon primers adopted here seem to function well to detect invertebrate prey from lizards' faeces.

The result of in silico PCR indicated that the original primer pairs generally enable to amplify more taxa compared with newly-designed primer pairs when amplicon lengths were ignored (Table S3). In the 16S rRNA primer sets, arthropods tended to be amplified with high taxonomic coverage (> 70% of examined genera), and others were relatively low efficiencies (13.5% and 44.9% of Mollusca, 71.0% and 57.6% of Annelida, and 0.2% and 0.8% of Chordata for 16S-short and 16S-long primer sets, respectively). In the COI primer sets, the original COI-long primer set could amplify all the examined invertebrate orders effectively (89.6% of Mollusca, 91.0% of Annelida, and 82.8% of Arthropoda). It was less efficient for Chordata than for invertebrates but still worked well (60.8%). The new COI-short primer set could amplify fewer number of sequences in all the examined taxa, and suppressed Trombidiformes (mites), Phasmatodea (stick insects), Blattodea (cockroaches), and Chordata very efficiently (0%–7.3%).

The blocking oligo did not completely erase the amplification of the lizards' DNA in any examined concentration but suppression was observed between any pairs of the examined concentration (Figure S2). To make the balance of suppression of the host DNA and prey DNA, we decided to use the blocking oligo by 5x concentration against the corresponding primer.

Table S1. Primer combinations used in the first PCR. Sequences indicated by abbreviations in parentheses were shown at the end of the table.

Primer set	Primer	Oligo sequence	Annealing temperature	Insert size (bp; mean±sd)	Insert size accepted (bp)	Reference
16S-short	IN16STK-1F	(SBS3)(N4,6,8)TGAACTCAGATCATGTAA	50°C	56.98±2.24	50–64	Kartzinel and Pringle (2015)
	16SAnn-R	(SBS12)(N4,6,8)TTGTGACCTCGATGTTGRCTT				This study
16S-long	IN16STK-1F	(SBS3)(N4,6,8)TGAACTCAGATCATGTAA	40°C	109.84±3.24	100–120	Kartzinel and Pringle (2015)
	IN16STK-1R	(SBS12)(N4,6,8)TTAGGGATAACAGCGTAA				Kartzinel and Pringle (2015)
COI-short	COI-Inve-F	(SBS3)(N4,6,8)TTATTACAGCARTWATTAATATACG	40°C	81.98±0.39	72–92	This study
	fwhR2n	(SBS12)(N4,6,8)GTRATWGCHCCDGCTARWACWGG				Vamos et al. (2017)
COI-long	fwhF2	(SBS3)(N4,6,8)GGDACWGGWTGAACWGTWTAYCCHCC	40°C	204.97±0.45	195–213	Vamos et al. (2017)
	fwhR2n	(SBS12)(N4,6,8)GTRATWGCHCCDGCTARWACWGG				Vamos et al. (2017)
	blocking-Goni-COI	GTTTACCCCCCATTAGCCGCAAACC-C3				This study
	SBS3	ACACTCTTTCCCTACACGACGCTCTTCCGATCT				
	SBS12	GTGACTGGAGTTCAGACGTGTGCTCTTCCGATCT				
	N4,6,8	equimolar mixture of NNNN, NNNNNN, and NNNNNNNN				

Table S2. The brief profile of bioinformatics procedure on the metabarcoding analyses. The upper section showed about all organisms detected and the lower showed about prey items only.

Locus	Filter-passed	ASVs	DB hits	Unique reference	Reference deficiency		Resolution problem of loci			
					Order	Higher	Genus	Family	Order	Higher
16S-short	2,480,780	448	287	73	102	52	13	21	23	3
16S-long	358,336	165	97	23	48	6	5	6	6	0
COI-short	1,163,444	455	180	58	72	27	6	8	7	1
COI-long	549,024	559	206	56	60	80	3	1	0	3

	No. samples prey detected	Unique reference	No. detected species	Reference deficiency	Resolution problem of loci		
				Order	Genus	Family	Order
16S-short	153	65	47	96	11	20	18
16S-long	58	21	17	44	3	6	5
COI-short	131	51	36	59	6	8	3
COI-long	53	19	15	26			

Table S3. The genus-level taxonomic coverage of the primer sets estimated by in silico PCR. The numbers of examined genera were shown for each genes followed by percentages of amplifiable genera for each primer set.

	taxid	16S tested	16S-S	16S-L	COI tested	COI-S	COI-L
Mollusca	6447	1198	13.5	44.9	3805	57.5	89.6
Gastropoda	6448	947	2.0	38.8	2937	70.6	94.6
Stylommatophora	6527	372	1.6	41.1	822	70.8	94.3
Annelida	6340	210	71.0	57.6	881	49.8	91.0
Clitellata	42113	86	68.6	67.4	293	69.6	95.2
Crassiclitellata	2803884	55	67.3	67.3	92	77.2	97.8
Arthropoda	6656	6479	76.5	78.9	37131	53.2	82.8
Arachnida	6854	511	72.6	80.0	3359	34.8	89.0
Araneae	6893	392	79.3	83.7	1775	45.7	89.0
Opiliones	43271	14	85.7	85.7	373	12.1	94.4
Sarcoptiformes	83137	24	41.7	58.3	348	15.2	82.5
Trombidiformes	83136	19	5.3	36.8	382	7.9	88.0
Chilopoda	7540	22	22.7	31.8	66	69.7	93.9
Lithobiomorpha	41362	3	66.7	100.0	11	63.6	90.9
Scolopendromorpha	41361	5	20.0	20.0	21	90.5	100.0
Diplopoda	7553	82	13.4	17.1	249	63.5	87.6
Glomerida	62004	0	-	-	12	16.7	100.0
Polydesmida	71419	67	3.0	3.0	102	74.5	92.2
Malacostraca	6681	491	80.9	85.1	2069	49.3	89.1
Amphipoda	6821	45	66.7	71.1	452	25.9	85.8
Isopoda	29979	22	18.2	72.7	276	39.9	85.1
Decapoda	6683	393	85.2	87.0	1120	62.0	91.9
Collembola*	30001	55	56.4	61.8	163	73.0	95.7
Insecta	50557	5166	79.2	80.3	30354	56.3	81.4
Archaeognatha	29994	9	100.0	100.0	19	89.5	94.7
Plecoptera	50622	60	93.3	95.0	211	81.0	94.8
Dermaptera	27434	6	100.0	100.0	47	55.3	89.4
Orthoptera	6993	278	86.0	88.5	800	69.0	86.4
Phasmatodea	7020	26	80.8	80.8	223	0.0	35.0
Blattodea	85823	191	88.5	90.1	322	0.0	94.4
Hemiptera	7524	755	86.0	86.1	3843	47.1	79.7
Psocoptera	30259	32	100.0	96.9	122	54.9	68.0
Hymenoptera	7399	302	82.5	85.1	3556	16.7	86.1
Coleoptera	7041	1821	75.0	74.6	7154	47.4	64.6
Lepidoptera	7088	631	94.9	95.2	8629	83.5	92.0
Diptera	7147	593	68.0	71.0	3357	80.3	86.7
Chordata	7711	5695	0.2	0.8	8160	0.1	60.8

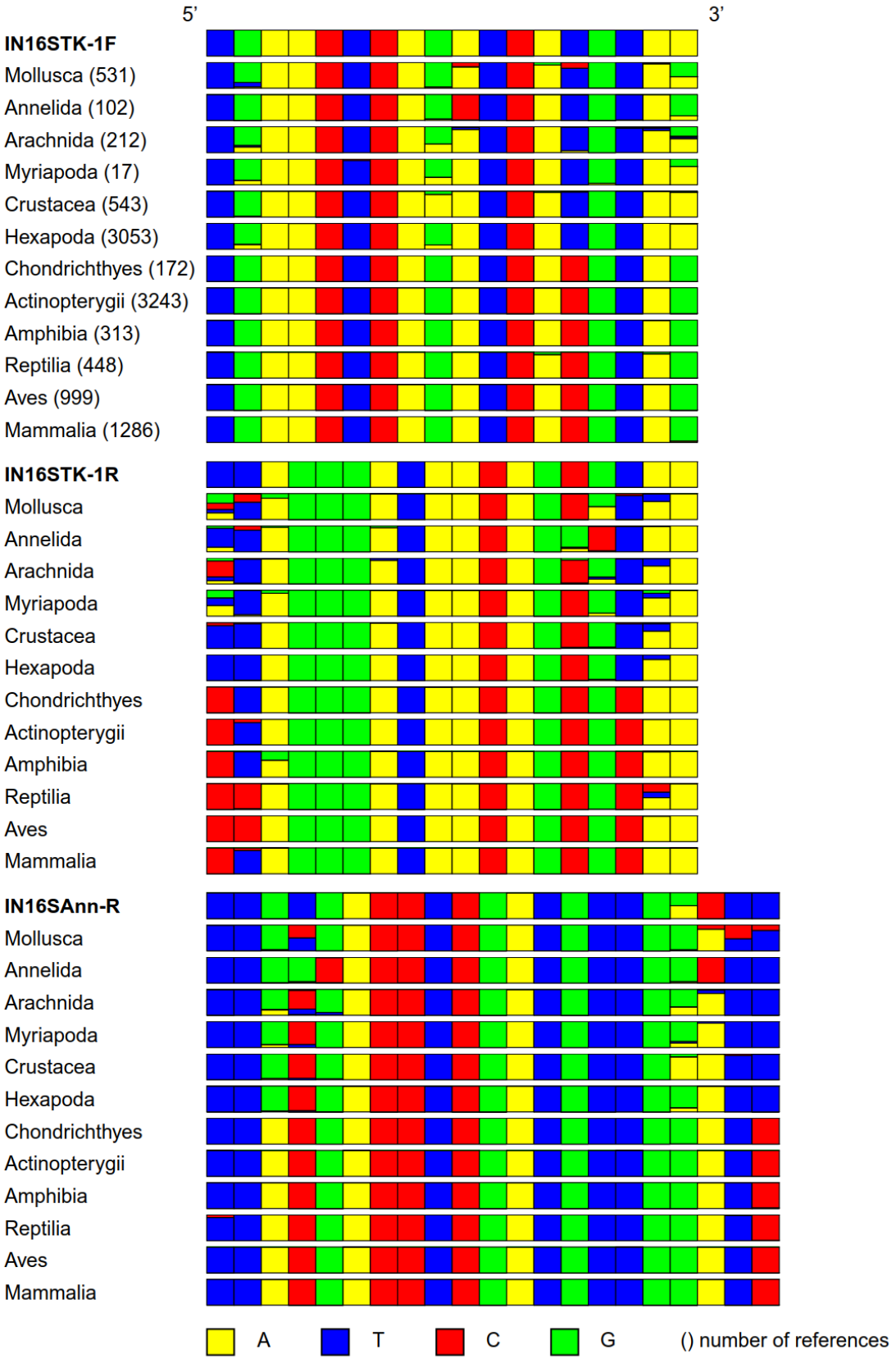

Figure S1. Comparisons of 16S rRNA primer sequences with the primer-binding sites of prey-candidate invertebrates and (probably non-prey) vertebrates. (Figure continues)

(Figure continued)

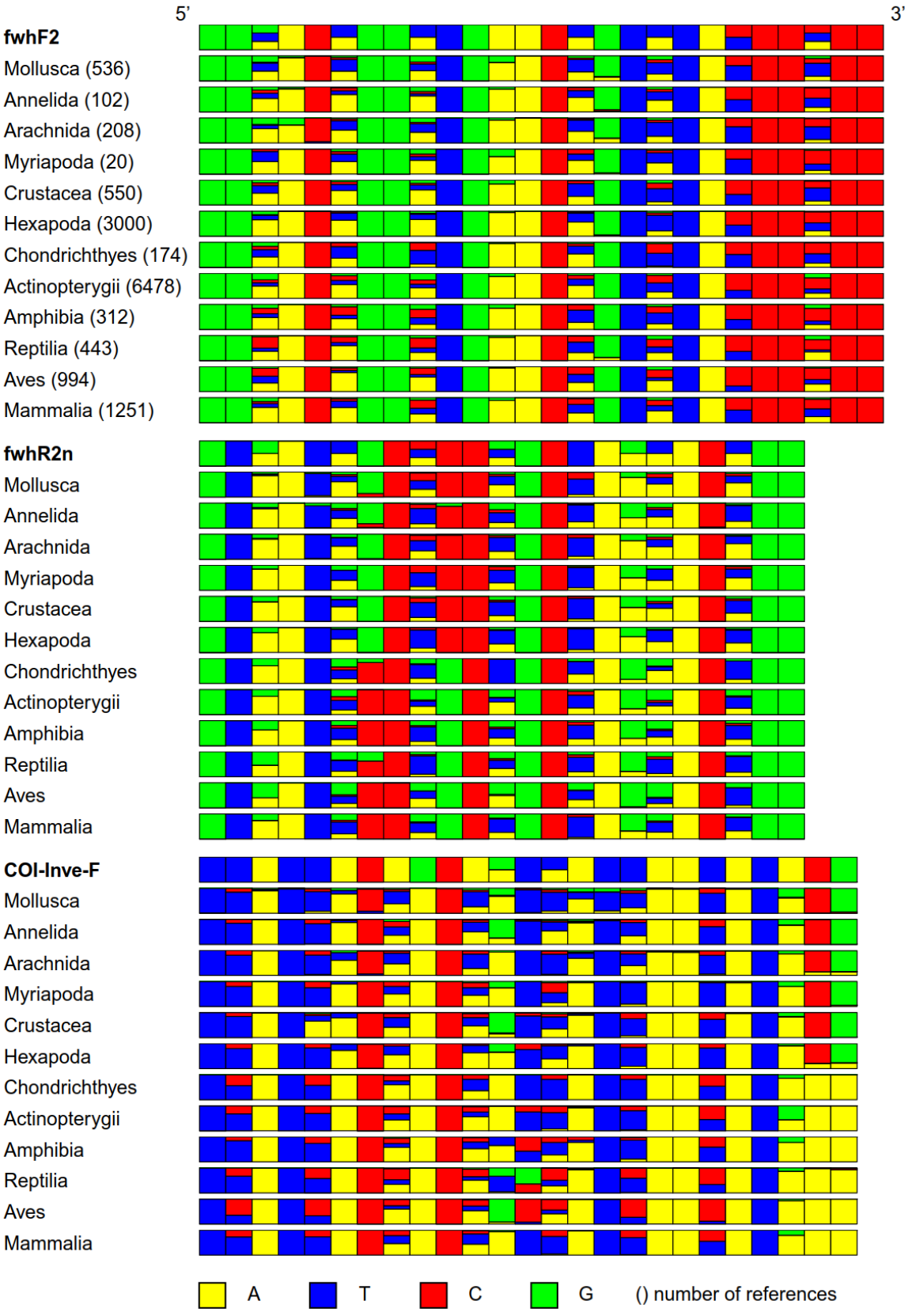

Figure S1. Comparisons of COI primer sequences with the primer-binding sites of prey-candidate invertebrates and (probably non-prey) vertebrates.

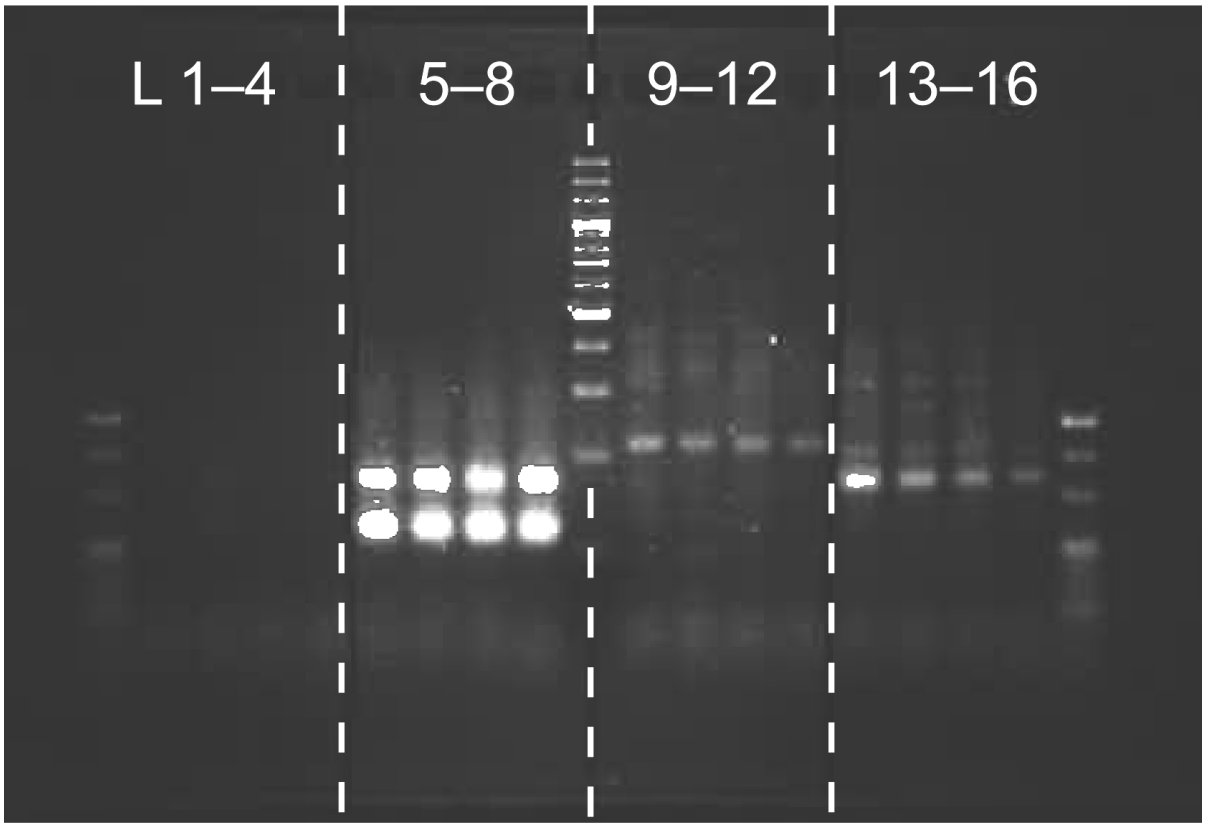

Figure S2. The agarose gel electrophoresis of PCR amplicons of COI gene with the various concentration of the blocking oligo. Lanes 1–4, 5–8, 9–12, and 13–16 are an earthworm, a centipede, an individual of G. kuroiwae from the southern part of Okinawajima, and that from the northern part of Okinawajima, respectively. Four lanes, for each organism, shows the results of 2x, 10x, 20x, and 40x concentration of the blocking oligo against the corresponding primer.