

ELECTRONIC SUPPLEMENTARY INFORMATION FOR
**NONLETHAL AMPHIBIAN SKIN SWABBING OF CUTANEOUS NATURAL
PRODUCTS FOR HPLC FINGERPRINTING**

Thomas P. Umile,^a Patrick J. McLaughlin,^b Kendall R. Johnson,^a Shaya Honarvar,^b
Alison L. Blackman,^c Elizabeth A. Burzynski,^a Robert W. Davis,^a Thais L. Teotonio,^c
Gail W. Hearn,^b Christine A. Hughey,^c Anthony F. Lagalante,^a and Kevin P.C.
Minbiole^{a*}

^a Department of Chemistry, Villanova University, Villanova, PA 19085 USA

^b Department of Biology, Drexel University, Philadelphia, PA 19104 USA

^c Department of Chemistry, James Madison University, Harrisonburg, VA 22807 USA

* corresponding author contact information:

email: kevin.minbiole@villanova.edu

tel: 610-519-4873

fax: 610-519-7167

SWABBING OF FROGS ON BOKO ISLAND

Swabbing of Bioko Frogs. Frog specimens were collected at night by hand from four different locations on Bioko Island. These sites ranged in elevation from 0-1400 m asl. Arena Blanca (0-100 m asl) consists of dense lowland tropical forest and brackish swamps, and represented 4% of total sampling effort. Risule and Moeri were comparable sites (400-700 m asl) consisting of a patchwork of disturbed and pristine submontane forest, and represented 85% of total sampling effort. Moka (1300-1400 m asl) is a mixture of montane tropical forest with large areas converted for agriculture, and represented 11% of total sampling effort. A total of 47 specimens were collected for swabbing across all sites. Frog cataloging information is listed below (Table S1).

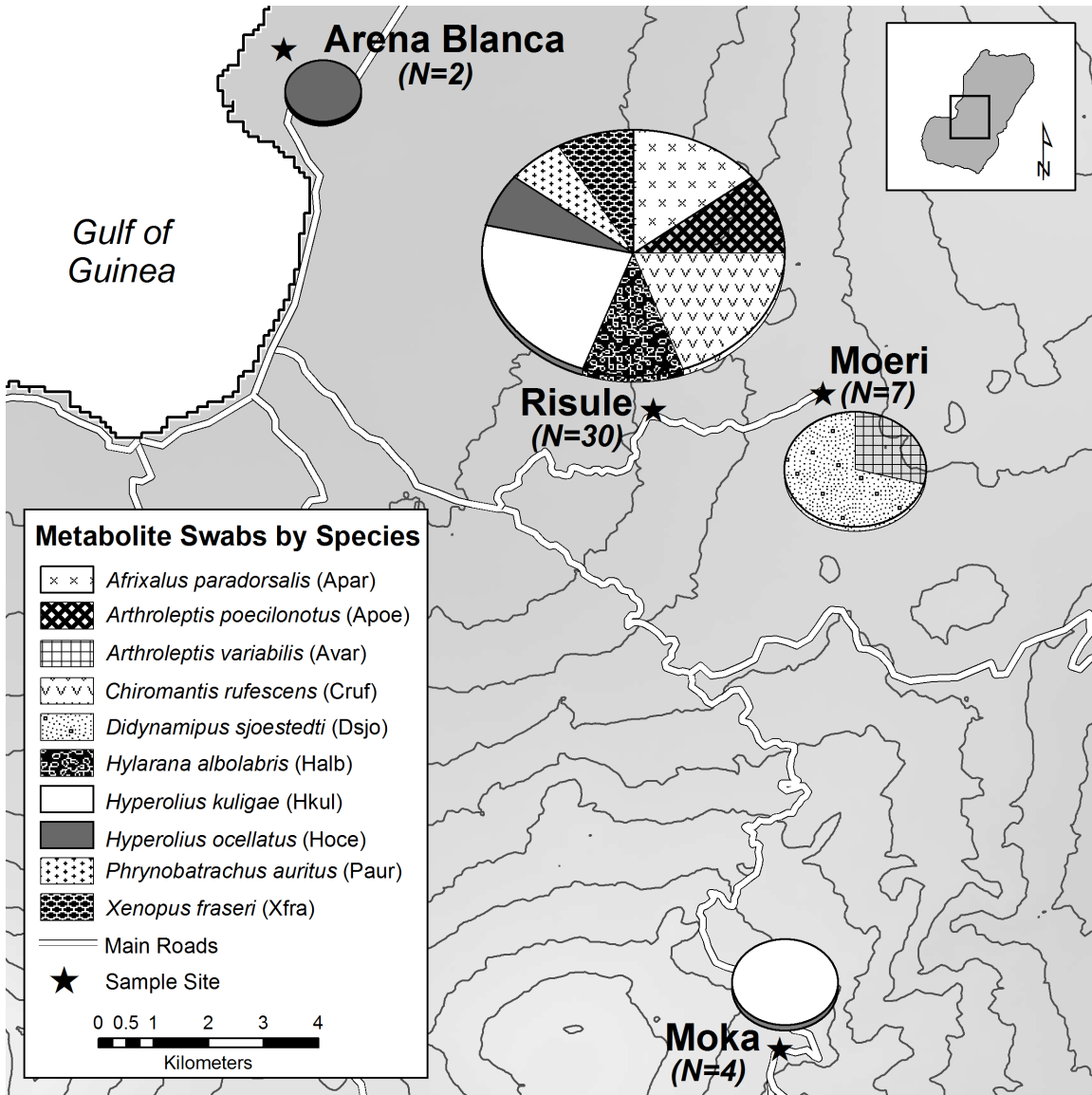


Fig. S1 Map of sampling sites on Bioko Island, Equatorial Guinea. The figure details the number and species of specimens from each site. Inset shows Bioko Island with focal area containing sample sites.

Family	Genus	Species	Map/PCO ID	Swabs	Sample Site	Body Size	Voucher Specimens	Healthy Release
Arthroleptidae	Arthroleptis	variabilis	Avar	2	Moeri	Medium	2	0
	Arthroleptis	poecilonotus	Apoe	3	Risule	Small	0	3
Bufonidae	Didynamipus	sjostedti	Dsjo	5	Moeri	Tiny	0	5
Hyperoliidae	Afrivalus	paradorsalis	Apar	5	Risule	Medium	0	5
	Hyperolius	kuligae	Hkul	9	5- Risule; 4- Moka	Small	5	4
	Hyperolius	ocellatus	Hoce	4	1- Arena Blanca; 2- Risuk	Small	4	0
Petropedetidae	Phrynobatrachus	auritus	Paur	2	Risule	Medium	0	2
Pipidae	Xenopus	fraseri	Xfra	3	Risule	Large	3	0
Ranidae	Hylarana	albolabris	Halb	4	Risule	Large	4	0
Rhacophoridae	Chiromantis	rufescens	Cruf	6	Risule	Large	0	6

Table S1. Summary of species sampled for cutaneous bacteria and small molecules on Bioko Island.

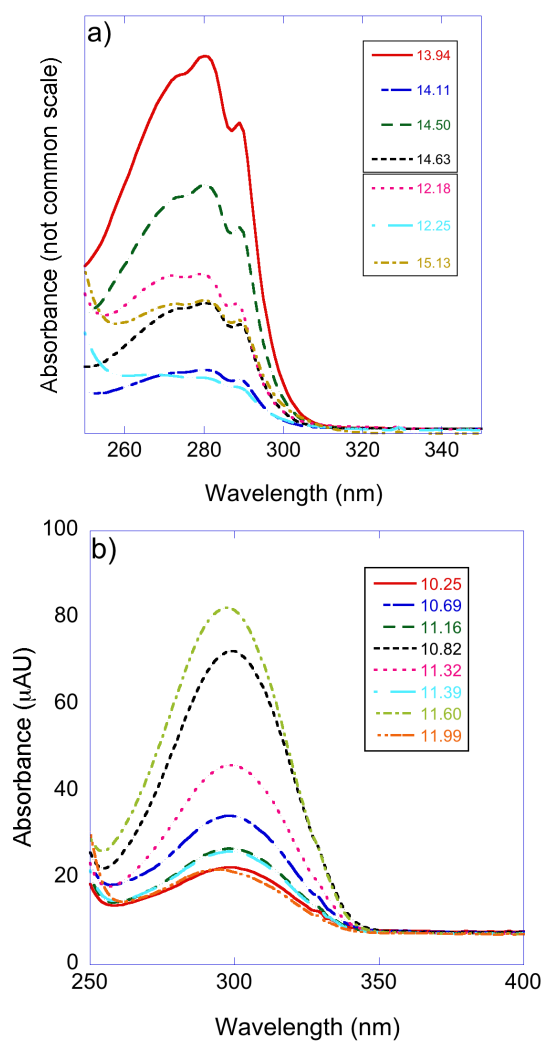


Fig. S2 Overlays of UV-Vis spectra of fingerprint compounds from a) *A. paradorsalis* and b) *D. sjostedti* (inset legends refer to the corresponding retention time in min)

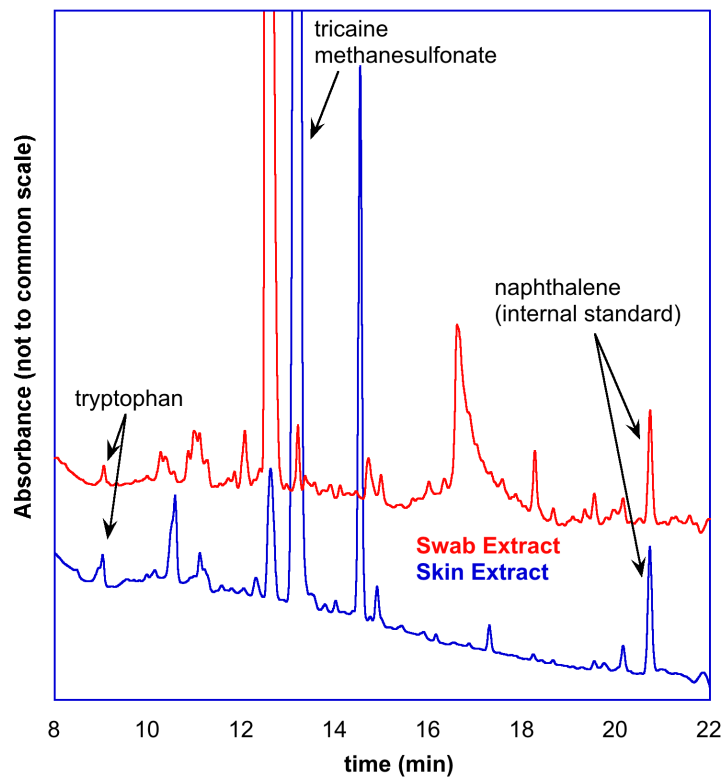


Fig. S3 Overlay of representative total wavelength chromatograms from swab extract and skin extract from a single frog specimen

Table S2 (*follows*) A comprehensive table of compounds found on each frog specimen, along with other specimen-specific notes. For each specimen, sample site and collection data are provided, along with notes regarding the location and/or microhabitat they were discovered in before capture. Where possible, sex was determined for each specimen. For each specimen, an "X" indicates a small molecule ("metabolite"), bacteria, or tissue sample was taken, while an "O" indicates no sample was taken. In the cases where two swab samples were taken, a starred X ("X*") indicates the sample that was collected first. For the "Specimen" column, an "X" indicates a museum specimen was preserved, "Healthy Release" indicates the specimen was released healthy to the site of capture within 24 hours, and "Skin Sample" indicates a skin tissue sample was taken and it was prepared as a museum specimen

Specimen No.	Species	Sampling Date	Notes	Sex	Location	Metabolite Swab	Skin Bacteria Swab	Tissue Sample	Specimen	Chromophore (nm)	
										Retention Time (min)	Chromophore (nm)
1	<i>Afrivalus paradorsalis</i>	9/19/12	Cement water tank	-	Risule	X	X*	O	Healthy Release	7.58	271
2	<i>Afrivalus paradorsalis</i>	9/19/12	Cement water tank	M	Risule	X*	X	O	Healthy Release	7.74	268
3	<i>Afrivalus paradorsalis</i>	9/19/12	Cement water tank	M	Risule	X	X*	O	Healthy Release	7.78	none
4	<i>Afrivalus paradorsalis</i>	9/19/12	Cement water tank	M	Risule	X*	X	O	Healthy Release	7.80	none
5	<i>Afrivalus paradorsalis</i>	9/19/12	Cement water tank	M	Risule	X	X*	O	Healthy Release	7.82	none
6	<i>Arthroleptis poecilnotus</i>	9/21/12	Fern on edge of trail	-	Risule	X	X*	O	Healthy Release	7.83	274, 351
7	<i>Arthroleptis poecilnotus</i>	9/21/12	Fern on edge of trail	-	Risule	X*	X	O	Healthy Release	7.86	268
8	<i>Arthroleptis poecilnotus</i>	9/21/12	Fern on edge of trail	-	Risule	X	X*	O	Healthy Release	7.98	280
9	<i>Arthroleptis sylvaticus</i>	9/16/12	Orange & Yellow Ventral Side	F	Moeri	X*	X	X	X	8.00	272, 357
10	<i>Arthroleptis variabilis</i>	9/16/12	Green	-	Moeri	X*	X	X	X	8.02	261
11	<i>Arthroleptis variabilis</i>	9/16/12	White w/ black pattern	-	Moeri	X	X*	X	X	8.08	none
12	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	F	Risule	X*	X	O	Healthy Release	8.09	274, 354
13	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	M	Risule	X	X*	O	Healthy Release	8.13	none
14	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	M	Risule	X*	X	O	Healthy Release	8.17	none
15	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	M	Risule	X	X*	O	Healthy Release	8.18	271
16	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	M	Risule	X*	X	O	Healthy Release	8.20	none
17	<i>Chiromantis rufescens</i>	9/19/12	Banana leaf above cement tank	M	Risule	X	X*	O	Healthy Release	8.21	269
18	<i>Didynamipus sjostedti</i>	9/16/12	Leaf litter alongside trail	F	Moeri	X*	X	X	X	8.25	none
19	<i>Didynamipus sjostedti</i>	9/16/12	Leaf litter alongside trail	M	Moeri	X	X*	X	X	8.29	271
20	<i>Didynamipus sjostedti</i>	9/16/12	Leaf litter alongside trail	F	Moeri	X	X*	X	X	8.33	270
21	<i>Didynamipus sjostedti</i>	9/16/12	Leaf litter alongside trail	M	Moeri	X*	X	X	X	8.38	280
22	<i>Didynamipus sjostedti</i>	9/16/12	Leaf litter alongside trail	M	Moeri	X	X*	X	X		
23	<i>Hylarana albolabris</i>	9/19/12	Cement water tank	F	Risule	X*	X	X	X		
24	<i>Hylarana albolabris</i>	9/19/12	Cement water tank	-	Risule	X	X*	X	X	+	+
25	<i>Hylarana albolabris</i>	9/19/12	Cement water tank	-	Risule	X*	X	X	X	+	+
26	<i>Hylarana albolabris</i>	9/19/12	Cement water tank	-	Risule	X	X*	X	X	+	+
27	<i>Hyperolius kuligae</i>	9/17/12	Metal drum, guarding nests	M	Moka	X	X*	X	Skin Sample		
28	<i>Hyperolius kuligae</i>	9/17/12	Metal drum, guarding nests	M	Moka	X*	X	X	Skin Sample		
29	<i>Hyperolius kuligae</i>	9/19/12	Cement water tank	M	Risule	X*	X	O	Healthy Release		
30	<i>Hyperolius kuligae</i>	9/19/12	Non-dichromatic coloration; yellow ventral side	F	Risule	X	X*	X	Skin Sample		
31	<i>Hyperolius kuligae</i>	9/19/12	Cement water tank	M	Risule	X*	X	O	Healthy Release		
32	<i>Hyperolius kuligae</i>	9/19/12	Cement water tank	M	Risule	X	X*	O	Healthy Release		
33	<i>Hyperolius kuligae</i>	9/19/12	Cement water tank	M	Risule	X*	X	O	Healthy Release		
34	<i>Hyperolius kuligae</i>	9/17/12	Metal drum, guarding nests	M	Moka	X	X*	X	Skin Sample		
35	<i>Hyperolius kuligae</i>	9/17/12	Outside metal drum	M	Moka	X*	X	X	Skin Sample		
36	<i>Hyperolius ocellatus</i>	9/21/12	Di-chromatic coloration	F	Risule	X	X*	X	X		
37	<i>Hyperolius ocellatus</i>	9/21/12	In amplexus with PJM436	M	Risule	X*	X	X	X		
38	<i>Hyperolius ocellatus</i>	9/21/12	Di-chromatic coloration	F	<i>Arena Blanca</i>	X*	X	X	Skin Sample		
39	<i>Hyperolius ocellatus</i>	9/21/12	In amplexus with PJM438	M	<i>Arena Blanca</i>	X	X*	X	Skin Sample		
40	<i>Leptopelis modestus</i>	9/19/12	Banana leaf above cement tank	-	Risule	X*	X	X	X		
41	<i>Leptopelis sp.</i>	9/17/12	Behind latrine at MWC	-	Moka	X	X*	O	Healthy Release		
42	<i>Phlyctimantis boulengeri</i>	9/19/12	Orange and black ventral mottling, black vocal sack	M	Risule	X	X*	X	X	+	+
43	<i>Phrynobatrachus auritus</i>	9/21/12	Fern on edge of trail	F	Risule	X*	X	O	Healthy Release		
44	<i>Phrynobatrachus auritus</i>	9/21/12	Fern on edge of trail	M	Risule	X	X*	O	Healthy Release		
45	<i>Xenopus fraseri</i>	9/19/12	Mud puddle near cement water tank	-	Risule	X	X*	X	X		
46	<i>Xenopus fraseri</i>	9/19/12	Mud puddle near cement water tank	-	Risule	X*	X	X	X		
47	<i>Xenopus fraseri</i>	9/19/12	Mud puddle near cement water tank	-	Risule	X	X*	X	X		

Specimen No.	274, 354
8.51	none
8.54	none
8.59	none
8.65	none
8.67	271
8.71	none
8.75	none
8.80	none
8.89	none
+ 9.01	279
9.04	none
9.06	280
9.26	288
9.27	282
9.28	260
9.39	none
+ 9.43	288
9.44	none
9.45	273
9.59	264
9.64	none
9.66	280
+ 9.68	none
+ 9.85	271
9.86	none
9.94	none
10.05	280
10.25	298
10.26	252
+ 10.37	267
10.37	272
10.38	none
10.43	none
10.47	279
10.47	none
10.54	324
10.69	300
10.70	none
10.77	none
10.80	none
10.82	none
10.82	298
10.84	none
+ 10.86	280
10.88	none
10.93	279
10.97	280
10.99	274
11.00	none
11.02	279
11.04	none
+ 11.08	280
11.09	none
11.12	none
11.13	280
11.16	298
11.17	none
11.20	none
11.28	279
11.32	298
11.34	none

Specimen No.	15.85	15.85	15.88	16.03	16.48	16.86	Sampling Date	Notes	Sex	Location	GPS	Metabolism Swai	Skin Bacteria Swi	Tissue Sample	Specimen
1	none						9/19/16	Cement water tank	-	Risule	X	X	O	Healthy Release	Healthy Release
2	275						9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
3							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
4							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
5							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
6							9/21/16	Fern on edge of trail	-	Risule	X	X	O	Healthy Release	X
7							9/21/16	Fern on edge of trail	-	Risule	X	X	O	Healthy Release	Healthy Release
8							9/21/16	Fern on edge of trail	-	Risule	X	X	O	Healthy Release	Healthy Release
9							9/16/16	Orange & Yellow Ventral Side	F	Moeri	X	X	X	X	Healthy Release
10							9/16/16	Green	-	Moeri	X	X	X	X	X
11							9/16/16	White w/ black pattern	-	Moeri	X	X	X	X	X
12							9/19/16	Banana leaf above cement tank	F	Risule	X	X	O	Healthy Release	Healthy Release
13							9/19/16	Banana leaf above cement tank	M	Risule	X	X	O	Healthy Release	Healthy Release
14							9/19/16	Banana leaf above cement tank	M	Risule	X	X	O	Healthy Release	Healthy Release
15							9/19/16	Banana leaf above cement tank	M	Risule	X	X	O	Healthy Release	Healthy Release
16							9/19/16	Banana leaf above cement tank	M	Risule	X	X	O	Healthy Release	Healthy Release
17							9/19/16	Banana leaf above cement tank	M	Risule	X	X	O	Healthy Release	Healthy Release
18							9/16/16	-	F	Moeri	X	X	X	Healthy Release	Healthy Release
19							9/16/16	-	M	Moeri	X	X	X	Healthy Release	Healthy Release
20							9/16/16	-	F?	Moeri	X	X	X	Healthy Release	Healthy Release
21							9/16/16	-	M	Moeri	X	X	X	Healthy Release	Healthy Release
22							9/16/16	-	M	Moeri	X	X	X	Healthy Release	Healthy Release
23							9/19/16	Cement water tank	F	Risule	X	X	X	X	X
24							9/19/16	Cement water tank	-	Risule	X	X	X	X	X
25							9/19/16	Cement water tank	-	Risule	X	X	X	X	X
26							9/19/16	Cement water tank	-	Risule	X	X	X	X	X
27							9/17/16	Metal drum, guarding nests	M?	Moka	X	X	X	Skin Sample	Skin Sample
28							9/17/16	Metal drum, guarding nests	M?	Moka	X	X	X	Skin Sample	Skin Sample
29							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
30							9/19/16	Non-dichromatic coloration; yellow ventral side	F	Risule	X	X	X	Skin Sample	Skin Sample
31							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
32							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
33							9/19/16	Cement water tank	M	Risule	X	X	O	Healthy Release	Healthy Release
34							9/17/16	Metal drum, guarding nests	M?	Moka	X	X	X	Skin Sample	Skin Sample
35							9/17/16	Outside metal drum	M?	Moka	X	X	X	Skin Sample	Skin Sample
36							9/21/16	<i>Di-chromatic coloration</i>	F	Risule	X	X	X	X	X
37							9/21/16	<i>In amplexus with Specimen 36</i>	M	Risule	X	X	X	X	X
38							9/21/16	<i>Di-chromatic coloration</i>	F	<i>Arena Blanca</i>	X	X	X	<i>Skin Sample</i>	<i>Skin Sample</i>
39							9/21/16	<i>In amplexus with Specimen 38</i>	M	<i>Arena Blanca</i>	X	X	X	<i>Skin Sample</i>	<i>Skin Sample</i>
40							9/19/16	Banana leaf above cement tank	-	Risule	X	X	X	X	X
41							9/17/16	Behind latrine at MWC	-	Moka	X	X	O	Healthy Release	Healthy Release
42							9/19/16	Unknown species; orange and black mottling, black vocal sack	M	Risule	X	X	X	X	X
43							9/21/16	Fern on edge of trail	F	Risule	X	X	O	Healthy Release	Healthy Release
44							9/21/16	Fern on edge of trail	M	Risule	X	X	O	Healthy Release	Healthy Release
45							9/19/16	Mud puddle near cement water tank	-	Risule	X	X	X	X	X
46							9/19/16	Mud puddle near cement water tank	-	Risule	X	X	X	X	X
47							9/19/16	Mud puddle near cement water tank	-	Risule	X	X	X	X	X