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Dear Editor,

We would like to thank the reviewer for thoughtful comments and efforts to review our manuscript entitled "Distinct Gut Microbiomes in Thai Patients with Colorectal Polyps." We have carefully considered each point raised by the reviewer and have made corresponding improvements to the article. We are committed to meeting your requirements and have highlighted all the updated sections in yellow in the revised manuscript. We provide point-by-point responses to these comments and suggestions as follows.

Comment

Point 1: Title, Abstract and Key Words reflect the main subject of the manuscript, however, many information is about colorectal cancer (CRC). Of course colorectal polyps progress to CRC and is closely linked. But I suggest to focus on polyps rather than CRC.

Response: We appreciate the reviewer for this suggestion. We have revised the abstract to focus on polyps.

Point 2: Background. The same remark goes to the introduction and there is too much about CRC - please focus on gut polyps. What is already known about polyps and their relation to the microbiome.

Response: We sincerely appreciate the valuable comments. We have revised the introduction in accordance with the comments.

Point 3: Methods. First the participants number should be included in the methods. Second how many were invited, how many were excluded and why. The inclusion and exclusion criteria are poorly described.

Response: Thank you for this suggestion. We have added the inclusion and exclusion criteria, as well as the number of participants, to the methods section and included the enrollment process in the Results section.

Point 4: Gut microbiome depends on diet. What about the diet in the patients or behavioral status?

Response: Thank you very much for your comment. The diet information of the participants was collected, but we could not access it. However, the screening project was conducted in Bangkok, and most participants lived in the central region. The major dietary patterns may not differ and will not be affected by differences in regional dietary habits.

Reference: Phoonlapdacha P, Tangshewinsirikul C, Phosuwattanakul J, Kittisakmontri K, Nitisinprasert S, Nakayama J, Prombutara P, Suthutvoravut U, Chongviriyaphan N. Gut microbiome profiles in Thai healthy pregnant women and its association with types of foods. *BMC Pregnancy Childbirth* 2022; **22**: 79-88 [PMID: 35093031 PMID: PMC8801080 DOI: 10.1186/s12884-022-04397-5].

Point 5: Results. As groups differ by age the gut microbiome may differ by age. So verify the methods and again inclusion criteria.

Response: Thank you for this important suggestion. As you rightly pointed out, it is true that the gut microbiome may differ by age. However, the CRC screening project aims to enroll older individuals aged 50-80 years old, covering a wide range of ages. Therefore, we investigated whether age affects the gut microbiome in each study group (CT, HP, and TA). We first analyzed microbial diversities within each age group (50-59, 60-69, and 70-79 years old). The differences in microbiome diversities were compared among study groups using the Kruskal-Wallis rank sum test. We analyzed the α -diversity between

two groups of participants: the CT vs. HP groups and the CT vs. TA groups. The outputs of the α -diversity calculations for each age group (50-59, 60-69, and 70-79 years old), using the Shannon, PD, Simpson, and Pielou indexes, are shown in Figures 1A and 1B. Then, the differences in microbial diversity between the CT and HP groups (Figure 2A), as well as between the CT and TA groups (Figure 2B), were analyzed using the Kruskal-Wallis rank sum test. The statistical test showed no significant difference in microbial diversity between the CT and HP groups, nor between the CT and TA groups, within each age group. This result indicates that age does not affect microbial diversity between these groups of participants.

A

id	Shannon PD	Simpson Pielou	Condition	age	age_rank	bmi	bmi_rank	is_poly	morphology	family_history	normal_weight	no	none	unknown
ct_01	3.3404818724115	7.411447634	0.946214628257887	0.728572642652654	Normal	57	group_50_59	19.9	normal_weight	no	none	unknown		
ct_02	3.75898514271245	9.506624807	0.961604960219479	0.747248092800627	Normal	66	group_60_69	22.13	normal_weight	no	none	no		
ct_03	3.14927809110181	7.020628254	0.951633720164609	0.6951606293815563	Normal	52	group_50_59	22.77	normal_weight	no	none	no		
ct_04	3.5993772525779	10.803186941	0.950713108367627	0.723240872200498	Normal	69	group_60_69	22.19	normal_weight	no	none	unknown		
ct_05	3.65979597931457	9.984191665	0.95168077914952	0.731381944479733	Normal	54	group_50_59	22.93	normal_weight	no	none	unknown		
ct_06	3.64595107397728	9.979205353	0.943897755829904	0.726678836453303	Normal	68	group_60_69	21.46	normal_weight	no	none	yes		
ct_07	3.3591238789314	7.63472776	0.93560096021948	0.695713671831157	Normal	58	group_50_59	21.45	normal_weight	no	none	yes		
ct_08	3.84958185808932	10.72209474	0.980776625514403	0.76509026959796	Normal	52	group_50_59	24.44	overweight	no	none	no		
ct_09	3.54304250913225	6.715422689	0.958787807956104	0.781759453767061	Normal	58	group_50_59	19.2	normal_weight	no	none	yes		
ct_10	3.41018647353751	8.059593472	0.952220400548697	0.747135479259051	Normal	55	group_50_59	20.9	normal_weight	no	none	unknown		
ct_11	3.67433653127841	8.213381576	0.955037355281207	0.767486761061905	Normal	66	group_60_69	19.56	normal_weight	no	none	yes		
ct_12	3.36923054182533	6.973004741	0.74323638957476	0.531731660039977	Normal	59	group_50_59	25.56	overweight	no	none	no		
ct_13	3.47430994754143	6.131350808	0.953188367626886	0.75120656568823	Normal	60	group_60_69	22.76	normal_weight	no	none	unknown		
ct_14	3.76705905355488	9.857327352	0.958672197530864	0.744096462185821	Normal	60	group_60_69	24.74	overweight	no	none	unknown		
ct_15	3.34092445341429	6.448275605	0.938003314128944	0.730302847352179	Normal	53	group_50_59	22.21	normal_weight	no	none	yes		
ct_16	3.2590542772685	6.073804741	0.74323638957476	0.531731660039977	Normal	59	group_50_59	21.23	normal_weight	no	none	unknown		
ct_17	3.4576660122188	9.124695748	0.92605475994513	0.707838884503343	Normal	56	group_50_59	23.7	overweight	no	none	unknown		
ct_18	3.47731916503542	8.964744823	0.940525849108368	0.706774928085748	Normal	52	group_50_59	19.22	normal_weight	no	none	no		
ct_19	3.42318724125852	5.796640956	0.818149596707819	0.552893229742249	Normal	53	group_50_59	22.23	normal_weight	no	none	unknown		
ct_20	3.7443337892832	12.519097919	0.946537108376227	0.75178199267844	Normal	52	group_50_59	18.37	normal_weight	no	none	yes		
ct_21	3.56576016843319	7.342620261	0.952769031590609	0.751488129222287	Normal	57	group_50_59	20.58	normal_weight	no	none	unknown		
ct_22	3.56545838661624	7.747614891	0.954294529492455	0.742408453696942	Normal	52	group_50_59	24.8	normal_weight	no	none	yes		
ct_23	3.22897473600282	7.900300424	0.928344493827161	0.670999799165616	Normal	53	group_50_59	20.3	normal_weight	no	none	unknown		
ct_24	3.99000505545654	10.14134138	0.9719615829904	0.803339150227695	Normal	53	group_50_59	24.31	overweight	no	none	unknown		
ct_25	3.69812798455128	8.486518695	0.957139008685711	0.763415598647835	Normal	53	group_50_59	24.4	overweight	no	none	unknown		
ct_26	3.66584085503436	9.423860889	0.95757514677641	0.75679470121566	Normal	60	group_60_69	19.13	normal_weight	no	none	unknown		
ct_27	3.44746308650789	7.044047961	0.950295122085048	0.742285298041299	Normal	61	group_60_69	18.61	normal_weight	no	none	unknown		
ct_28	3.28232427320228	4.898571114	0.91334937229081	0.709033738963765	Normal	53	group_50_59	21.6	normal_weight	no	none	unknown		
ct_29	3.79613613987927	8.954547197	0.965453673525377	0.7875347336484	Normal	52	group_50_59	22.03	normal_weight	no	none	no		
ct_30	3.447372437605929	7.778594879	0.951647180902646	0.729578400816574	Normal	62	group_60_69	19.98	normal_weight	no	none	yes		
ct_31	3.80121950073749	12.823746873	0.95913426611797	0.724452460758833	Normal	55	group_50_59	19.57	normal_weight	no	none	no		
ct_32	3.53515160878618	11.377633839	0.92757238957476	0.712323651486354	Normal	65	group_60_69	21.79	normal_weight	no	none	no		
ct_33	3.94508522902047	9.80712795	0.851478507544582	0.684095615782515	Normal	56	group_50_59	19.531	normal_weight	no	none	no		
ct_34	3.43882945607225	5.242820825	0.95540446392318	0.760502044512727	Normal	66	group_60_69	21.94	normal_weight	no	none	unknown		
ct_35	3.80713512197724	10.42314348	0.95502814814815	0.739609365124378	Normal	55	group_50_59	24.65	overweight	no	none	no		
ct_36	3.95426493009827	9.687434618	0.970796104252401	0.809835211993144	Normal	55	group_50_59	22.19	normal_weight	no	none	no		
ct_37	3.33978674384738	8.086822086	0.92111502808584	0.686118788719715	Normal	52	group_50_59	18.55	normal_weight	no	none	no		
ct_38	3.09965249799836	8.481690864	0.907708937141209	0.646327746912398	Normal	50	group_50_59	26	overweight	no	none	unknown		
ct_39	3.0748923994109	5.846533839	0.918935111111111	0.690312639673088	Normal	53	group_50_59	21.45	normal_weight	no	none	unknown		
ct_40	3.0748923994109	5.846533839	0.918935111111111	0.646327746912398	Normal	53	group_50_59	21.45	normal_weight	no	none	unknown		
hp_01	3.93098946307932	6.374329893	0.895137514403292	0.648599138663502	hyperplastic_poly	64	group_60_69	18.56	normal_weight	yes	sessile	yes		
hp_02	4.2198360853579	10.716731968	0.97778454209877	0.82450895994394	hyperplastic_poly	63	group_60_69	23.18	overweight	yes	sessile	yes		
hp_03	3.93308423252728	5.508627394	0.901848952529293	0.66761592477134	hyperplastic_poly	56	group_50_59	25.97	overweight	yes	none	no		
hp_04	3.69626320890022	12.168001191	0.92307134662514	0.692093570869685	hyperplastic_poly	66	group_60_69	21.87	normal_weight	yes	none	no		
hp_05	3.78407253833263	10.956282666	0.96134633744856	0.753342465578812	hyperplastic_poly	58	group_50_59	22.57	normal_weight	yes	sessile	no		
hp_06	3.430121265425616	11.946368642	0.961351561042524	0.751386290345078	hyperplastic_poly	60	group_60_69	32.47	obese	yes	sessile	no		
hp_07	3.87482836159971	9.498621466	0.9639511859808	0.77869793463682	hyperplastic_poly	63	group_60_69	23.34	overweight	yes	sessile	no		
hp_08	3.895250891030318	8.96821105	0.95138587626886	0.78959535914642	hyperplastic_poly	63	group_60_69	22.76	normal_weight	yes	none	unknown		
hp_09	3.39511124476501	8.794021009	0.93019459396433	0.71422043303544	hyperplastic_poly	53	group_50_59	26.23	overweight	yes	none	unknown		
hp_10	3.5069308841264	8.110218351	0.951050139917695	0.741832725153458	hyperplastic_poly	54	group_50_59	27.7	obese	yes	sessile	yes		
hp_11	3.139388451927	6.181666134	0.90095299039781	0.683502960440052	hyperplastic_poly	59	group_50_59	23.7	overweight	yes	none	unknown		
hp_12	3.44236641707546326	7.483482215	0.948096246031358	0.741295090524236	hyperplastic_poly	71	group_70_79	31.83	obese	yes	sessile	no		
hp_13	3.5801110203958	6.624858734	0.783340444444444	0.548980789771476	hyperplastic_poly	65	group_60_69	24.93	overweight	yes	sessile	yes		
hp_14	3.26013518014026	7.383276893	0.93424126611797	0.717570691838616	hyperplastic_poly	69	group_60_69	31.39	obese	yes	none	no		
hp_15	3.430121265425616	11.946368642	0.961351561042524	0.751386290345078	hyperplastic_poly	57	group_50_59	25.92	overweight	yes	sessile	no		
hp_16	3.6811806804875	9.143941303	0.95076551168724	0.748346363086579	hyperplastic_poly	63	group_60_69	22.94	normal_weight	yes	flat	yes		
hp_17	3.404211481983542	8.089112025	0.94522108179096	0.72851187341408	hyperplastic_poly	62	group_60_69	21.37	normal_weight	yes	flat	yes		
hp_18	3.14559639026549	7.733832867	0.926395017832647	0.68158485765548	hyperplastic_poly	61	group_60_69	18.67	normal_weight	yes	flat	yes		

B

id	Shannon PD	Simpson Pielou	Condition	age	age_rank	bmi	bmi_rank	is_poly	morphology	family_history	normal_weight	no	none	unknown
ct_01	3.3404818724115	7.411447634	0.946214628257887	0.728572642652654	Normal	57	group_50_59	19.9	normal_weight	no	none	unknown		
ct_02	3.75898514271245	9.506624807	0.961604960219479	0.747248092800627	Normal	66	group_60_69	22.13	normal_weight	no	none	no		
ct_03	3.14927809110181	7.020628254	0.951633720164609	0.6951606293815563	Normal	52	group_50_59	22.77	normal_weight	no	none	no		
ct_04	3.5993772525779	10.803186941	0.950713108367627	0.723240872200498	Normal	69	group_60_69	22.19	normal_weight	no	none	unknown		
ct_05	3.65979597931457	9.984191665	0.95168077914952	0.731381944479733	Normal	54	group_50_59	22.93	normal_weight	no	none	unknown		
ct_06	3.64595107397728	9.979205353	0.943897755829904	0.726678836453303	Normal	68	group_60_69	21.46	normal_weight	no	none	yes		
ct_07	3.3591238789314	7.63472776	0.93560096021948	0.695713671831157	Normal	58	group_50_59	21.45	normal_weight	no	none	yes		
ct_08	3.84958185808932	10.72209474	0.980776625514403	0.76509026959796	Normal	52	group_50_59	24.44	overweight	no	none	no		
ct_09	3.54304250913225	6.715422689	0.958787807956104	0.781759453767061	Normal	58	group_50_59	19.2	normal_weight	no	none	yes		
ct_10	3.41018647353751	8.059593472	0.952220400548697	0.747135479259051	Normal	55	group_50_59	20.9	normal_weight	no	none	unknown		
ct_11	3.67433653127841	8.213381576	0.955037355281207	0.767486761061905	Normal	66								

A

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Kruskal-Wallis rank sum test

data: Shannon by age_rank
Kruskal-Wallis chi-squared = 1.7872, df = 2, p-value = 0.4092

Kruskal-Wallis rank sum test

data: PD by age_rank
Kruskal-Wallis chi-squared = 1.3533, df = 2, p-value = 0.5083

Kruskal-Wallis rank sum test

data: Simpson by age_rank
Kruskal-Wallis chi-squared = 1.3724, df = 2, p-value = 0.5035

Kruskal-Wallis rank sum test

data: Pielou by age_rank
Kruskal-Wallis chi-squared = 1.6301, df = 2, p-value = 0.4426

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B

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Kruskal-Wallis rank sum test

data: Shannon by age_rank
Kruskal-Wallis chi-squared = 1.4334, df = 2, p-value = 0.4884

Kruskal-Wallis rank sum test

data: PD by age_rank
Kruskal-Wallis chi-squared = 1.3523, df = 2, p-value = 0.5086

Kruskal-Wallis rank sum test

data: Simpson by age_rank
Kruskal-Wallis chi-squared = 1.6939, df = 2, p-value = 0.4287

Kruskal-Wallis rank sum test

data: Pielou by age_rank
Kruskal-Wallis chi-squared = 2.0543, df = 2, p-value = 0.358

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Figure 2 The difference in microbial diversity between (A) the CT and HP groups, as well as between (B) the CT and TA groups in R software.

Point 6: Discussion. The discussion is broad, however again many times authors discuss CRC not polyps.

Response: We appreciate your kind comment. We have added more detailed about polyps to the discussion.

Point 7: Illustrations and tables. Some figures are small and hardly to interpret. I understand that methods unable authors better visualization but I expect better description in the text in the section results.

Response: We sincerely appreciate the valuable comments. We have resubmitted the figures with higher quality, as shown in Figures 5 and 6, to make them more clearly correspond to the text in the results section.

Point 8: Please add strength and limitation of the study.

Response: Thank you for this important suggestion. We have added the strength and the limitation to the discussion.