# Chapter 5: Using transcriptomics to investigate evolution and toxicology in *Gambierdiscus*. <sup>1</sup>

Key words: Gambierdiscus, ciguatoxin, pan-transcriptome

# 1 Abstract

Species of the genus Gambierdiscus produce Ciguatoxins (CTXs), the causative agent of ciguatera fish poisoning, a potentially debilitating seafood borne illness. Species of Gambierdiscus possess very large genomes, 32 - 35 Gbp, and, as with other dinoflagellates, possess unique genomic characteristics, such as highly repetitive and complex genome architecture. The exact toxins produced by species of Gambierdiscus remain largely unclear. It has been verified using LCMS on multiple strains that the species Gambierdiscus polynesiensis produces anaologs of CTXs. Other species appear to produce maitotoxins, gambierol, and other uncharacterised toxins. An understanding of the evolution of Gambierdiscus and their toxins requires information regarding their genetics. Transcriptomic sequencing is a feasible alternative to genome sequencing. In this study, we generated de novo RNA-seq libraries for Gambierdiscus polynesiensis, Gambierdiscus carpenteri, Gambierdiscus cf. silvae and Gambierdiscus lapillus, compared these to a previously sequenced Gambierdiscus australes, to discover a set of core genes shared by all species. We present a Gambierdiscus core transcriptome, which might be used to investigate candidate genes related to toxin production.

#### To do:

- re-structure as per Tim's comments
- incl Sammy's comments

# 2 Introduction

The challenge of protist de novo sequencing projects lies in assessing the adequacy and completeness of sequencing as well as library processing and assembly methods employed, without a well annotated reference. This issue is particularly prevalent in dinoflagellates, whose expansive and complex genetics tend to be a barrier to genomic sequencing. As an alternative to wrangling with dinoflagellate genomes, transcriptomes are used as to explore their genetics. This is due to the apparent presence of uncharacterized genetic mechanism(s) which seem to leave protein synthesis regulation to the post-transcriptional stage, thus with the effect that mRNA gives an approximation of genomic content. An indication of these regulatory mechanisms comes from a number of direct previous observations. Harke et al. (2017) cultured Provocentrum minimum and Alexandrium monilatum under stress conditions by severely limiting nitrogen as well as phosphorous availability. The cultures showed significant biochemical changes (e.g. growth rate, particulate organic carbon and particulate carbohydrates content) between the control and stress conditions at time of harvest, yet change in transcriptome expression was minimal, between 0.1 to 1 % depending on stressor and species used [11]. While the difference in biochemical changes was not captured by mRNA profiling of the cultures, the study did not include a protein expression observation to verify a difference in expression despite a static pool of mRNA availability [11]. As these organisms are relatively difficult to culture and extract RNA, until the MMEPTSP the number of marine eukaryotic transcriptomes was sparse. When searching for Gambierdiscus on NCBI's SRA database 5 relevant projects were found in addition to the MMETSP results (searched on November 10, 2018). These sequencing projects covered two strains of G. polynesiensis, as well as for G. australes and G. excentricus. The fifth project focused on the bacterial associations of G. caribaeus and G. carolinianus. Broadening the search to the order gonyalacales yielded a further 19 projects, including another on bacterial associates as well as 3 projects on Azadinium and Crypthecodinium, which are arguably not part of the gonyaulacales (see chapter 4). Searching for members of the phylum dinoflagellates calls a further 84 projects. Despite their ecological relevance for nutrient cycling, DMSP production, coral symbiosis and neurotoxin production (for a review see [30]), the paucity of sequencing data, even with the MMETSP dataset, is evident. This is further confounded to a large proportion of dinoflagellate transcriptomes

sharing no known similarity to other described proteins or domains compared to known databases. When compared to NCBI's nr database, the proportion of contigs with no known match was 60 % for Azadinium spinosum [27], over 50 % for G. australes & G. belizeanus [19], 57.9 % for G. excentricus [18], 63 % for G. polynesiensis [18, 31], and 55 - 57 % for Karenia brevis [38].

The concept of a reference genome, or transcriptome, allows for direct comparison of genome/transcriptome sequencing to a standard. However sequencing further genomes in bacteria reveled a large transitory subset of genetic content, with the conclusion that a single strain based reference would be inadequate for capturing a large proportion of the species' genetic diversity [41, 42]. An alternative approach to a reference genome was proposed - that of a core-genome common to all strains, and a pan-genome which is transitory. An extrapolation of this study by Tettelin et al. (2005), which showed that 1.5 % of the genome was novel between 8 strains of Streptococcus predicted based on mathematical models that for every new strain sequenced 22 novel genes are predicted to be discovered [26]. Since then the core- and pan-genome, or transcriptome, concept has been adopted for eukaryotes also, with the realisation that the transient genomic content holds true when multiple strains of a species are sequenced (e.g. [14, 24, 32, 33, 35, 39]). Further to exploring the shared and transient genetic components within a genus, pan and core analyses have been conducted for higher taxonomic levels, commonly within genus though also at much higher levels, such as the gene frequency of Eubacteria within the super kingdom inter-species pan and core analysis have also been conducted [12, 14, 20, 22, 42].

Five transcriptomes of Gambierdiscus were compared in this study with the aim of providing a pan-transcriptomic baseline Gambierdiscus de novo transcriptome sequencing, which can be expanded and refined in future studies. The taxa originated from two locations in Australia (Merimbula, NSW, and Heron Island, QLD) and Rarotonga in the Cook Islands (Table 1). All of the five species have been implicated in MTX production via bioassays, while G. carpenteri did not register for CTX-like activity in a bioassay [23]). The toxin profiles registered all species apart from G. carpenteri as an MTX producer, while only G. polynesiensis had a confirmed CTX production profile (Table 1) This study revealed a set of core-transcripts shared by all taxa as well as a subset of species specific, unique portion of the transcriptome. The results in this study

could provide an avenue of investigation of quering the expression differences between toxic and non-toxic species of Gambierdiscus.

Table 1: Gambierdiscus species transcriptomes used in this study along with their toxicity, toxin profile, accession numbers and source. Where possible, information is strain specific & otherwise denoted with \*

Species	G. australes	G. carpen-	G. lapillus	G. polyne-	G. cf. silvae
		teri		siensis	
Strain	CAWD149	UTSMER9A	HG4	CG15	HG5
TranscriptomeMMETSP		chapter 4	chapter 4	chapter 4	chapter 4
source					
Accession	MMETSP076	6 SRR6821720	SRR6821722	SRR6821723	SRR6821721
ID					
Isolation lo-	Rarotonga,	Merimbula,	Heron	Rarotonga,	Heron
cation	cation Cook Islands		Island, Cook Islands		Island,
	(2007)		Australia	(2014)	Australia
			(2014)		(2014)
Toxin pro-	CTX -ve;	CTX -ve;	CTX -ve;	CTX +ve;	CTX -ve;
file (LC-	MTX +ve	MTX -ve	MTX +ve	MTX +ve	MTX +ve
MS/MS)					
Toxicity via	CTX +ve;	CTX -ve;	$CTX + ve^*;$	$CTX + ve^*;$	$CTX + ve^*;$
bioassay	MTX N/A	MTX +ve	MTX +ve*	MTX +ve*	MTX +ve*
References	[17, 29, 36]	[23]	[21, 23]	this study,	[21, 23]
				[?]	

# 3 Methods

Scripts used for this project are available on Github under hydrahamster/pan-tran. Venn diagrams were created with InteractiVenn [13].

# 3.1 Transcriptome acquisition

Species of *Gambierdiscus* used in this chapter are summarized in Table 1. Toxicity and toxin profile reports are specific to the strains used as inter-species variation in toxin production was recently reported [23, 37], unless noted otherwise. The *G. polynesiensis* toxin profile was elucidated by Tim Harwood at the Cawthron institute with the same methodology as for *G. lapillus* in **Capter 2**. Seq libraries were assembled as per the transcriptome assembly subsection in the methods of **chapter 4**, without diginorm.

# 3.2 Spliced leader search

The spliced leader sequences reported by Zhang et al. (2007) were used to build a hmmer library [?]. The transcriptome assemblies were searched with the dinoSL hmmer library to investigate for spliced leader presence. All clusters were searched for membership of one or more contigs with a dinoSL.

# 3.3 Homolog clustering

Cd-hit was used to cluster highly similar transcripts to reduce redundancy with the flags -T 10 -M 5000 -G 0 -c 1.00 -aS 1.00 -aL 0.005 as shown by Cerveau and Jackson (2016) [3, 8]. Transdecoder was use to predict coding regions on the clustered nucleotide sequences [10]. Protein clusters were annotated with Interproscan v5.27 with local lookup server [34]. Protein clusters were processed to include the species of origin instead of the TRINITY tag and concatenated for input to get\_homologues [43]. The -t 0 flag was used for get\_homologues to acquire all possible clusters even with only one species representative, and -G for the OMCL algorithm. The resulting core-, softcore- and unique-clusters were matched with their interpro annotations and GO terms were queried with GOSUM

against the basic Gene Ontology (GO) database [1, 4, 15]. GOSUM was run at levels 1 and 2 of GOs with the go-basic GO reference.

# 3.4 Ketosynthase domain search

The transcriptome assemblies were queried for the ketosynthase (KS) active domain of the polyketide synthase (PKS) enzyme using hmmer [6] with libraries developed for this project. The contigs which were identified to contain an active domain were then searched for within the clusters to identify how the active domains clustered; and the assemblies were searched to compare KS abundance between species. The KS domains found were aligned with MUSCLE with a maximum of 8 iterations [7]. Maximum likelihood (ML) inference was run with the KS alignments using RaxML [40] with the -PROTGAMMAILGF flags on the University of Technology Sydneys High-performance computing cluster (HPCC)

# 4 Results

# 4.1 Overview of the transcriptomes

The progression of clustering and annotation results per transcriptome can be found in Table 2. A total of 287,546 clusters were found across all five species (Fig. 1).

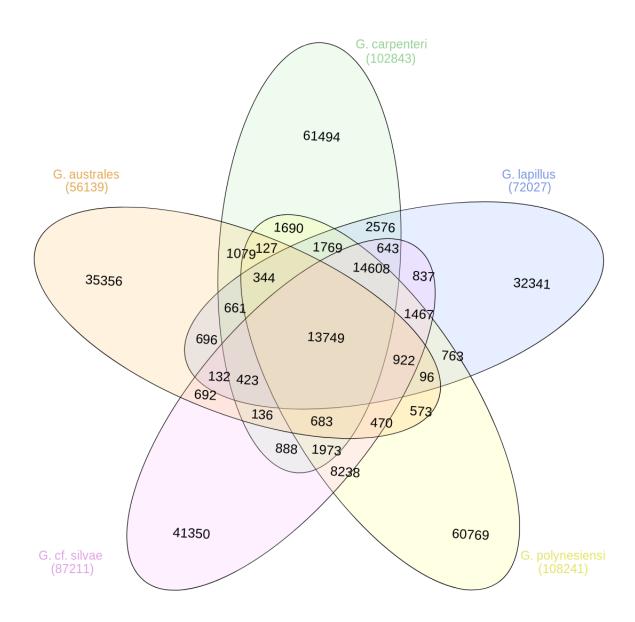


Figure 1: Venn diagram of species distribution across clusters.

Table 2: Progression of clusters found in each *Gambierdiscus* transcriptome during

processing.

processing.	Г	T	T		T
Species	G. aus-	G. carpenteri	G. lapillus	G. polyne-	G.  cf.  sil-
	trales			siensis	vae
Contigs	102,863	263,829	148,972	270,315	191,224
Spliced leader	304	683	232	1,570	1,524
contigs					
Nucleotide	102,861	263,743	148,966	270,265	191,205
clusters (cd-					
hit)					
Predicted	63,299	180,568	111,862	176,290	132,688
coding regions					
(Transde-					
coder)					
Contigs anno-	131,970	334,737	225,324	225,324	254,844
tated (Inter-					
pro Scan)					
Core-	13,750	13,750	13,750	13,750	13,750
transcriptome					
clusters					
Softcore-	2,372	16,058	16,297	16,557	16,636
transcriptome					
clusters					
Unique clus-	35,356	61,494	32,341	60,769	41,350
ters					

**Tim:** It seems kinda conspicuous that the unique clusters of *G. carpenteri & G. poly* are almost twice the number of *G. lapillus* and *G. silvae*, the first two were sequenced together with 150bp read length while the other two had 75bp read length during sequencing. Does this seem odd to you too?

#### 4.1.1 Core transcritome

A set of core genes common to all five species of Gambierdiscus were found. This set consisted of 13,750 amino acid clusters (Table 2) of which 45 % were annotated with GO terms (Suppl. table 5 & 6). The highest number of contigs in any core cluster was 180 cluster of unknown function with 23, 45, 32, 31 and 49 from G. australes, G. carpenteri G. lapillus, G. polynesiensis and G. cf. silvae respectively. Twelve of the core clusters contained 100 or more contigs, of which 3 were unannotated. The predicted protein coding regions for the other nine clusters, in descending order of contig numbers: an enzyme with catalytic activity involved in metabolic process; a calcium binding transmembrane transport channel; a protein involved in calcium binding; a protein binding enzyme; a domain for unspecified protein binding; an enzyme with O-glucosyl hydrolase activity involved in carbohydrate metabolic process; membrane bound ion transporter with cation channel activity & ionotropic glutamate receptor activity; a transmembrane transporter with voltage-gated calcium channel activity; and calcium ion binding transmembrane ion transporter. A total of 3,943 core clusters contained 10 or more contigs, so 71.32~% of the total core clusters consisted of less than 10 contigs. The majority of clusters fell within metabolic processes, cellular processes and catalytic activity with %, % and % of annotated clusters respectively. Tim - so adding up the lvl1 gosum counts for bio, cell and molec doesn't add up to the total annotated clusters... am I correct in thinking that this is because annotations can go to other functions too?

#### 4.1.2 Softcore transcriptome

A softcore with 4 out of the five *Gambierdiscus* species examined was identified. The softcore consisted of an additional 16,980 clusters (Table 2) of which 48 % were annotated (Suppl. table 5 & 6). The most prolific cluster in the softcore contained 163 contigs with unknown function, where *G. carpenteri G. lapillus*, *G. polynesiensis* and *G. cf. silvae* contained 50, 42, 41 & 30 contigs respectively. A further 5 clusters contained

more than 100 contigs, four of which had GO annotations. Of the six clusters with over 100 contigs, none had representatives contigs from *G. australes. G. australes* was absent from 86 % of the softcore clusters. In descending order of contigs, they matched to: a protein involved in selective protein binding; a protein involved in actin binding; a protein involved in calcium binding; and a protein with cysteine-type peptidase activity. Of the softcore, 14,035 clusters contained 10 or more contigs.

#### 4.1.3 Unique part of the transcriptome

Clusters with single species representatives, or the pan-transcriptome to the five *Gambierdiscus* species examined, numbered 231,310 clusters. Of the unique clusters, only 15.23 % of clusters were annotated. Single species clusters from *G. australes*, *G. carpenteri G. lapillus*, *G. polynesiensis* and *G.* cf. *silvae* numbered 35,356, 62,494, 32,341, 60,796 & 41,350 clusters respectively (Table 2). The highest number of contigs in a unique cluster were 37, found in two clusters from *G. carpenteri*. One of these was annotated for RNA and metal ion binding activity. Of the unique clusters, 83.1 % contained only one contig and 97.8 % of clusters have 5 contigs or less.

#### 4.1.4 Comparison of gene ontology annotations.

The GOs were split up into the three functional groups defined by the consortium: 1) Molecular processes (Figs. 4, 7, 10 & 13) defined as biochemical or a macromolecule directly interacting with other molecules; 2) Cellular components (Figs. 2, 6, 9 & 12) defined by the location within the cell where a molecular process takes place; and 3) Biological process (Figs. 3, 5, 8 & 11) which is defined as a molecular machinery participating in the execution of the cell's genetic programming, e.g. cell division. GO basic is structured in a hirachical manner, with parent and child terms where child terms are more specific than parent terms. For a general overview of functions present in each transcriptome, level 1 GO terms were elucidated (Figs. 3, 2 4, 8, 9 & 10). A more in depth query of the functions present in each transcriptome was conducted with a GO search of the child terms at level 2 (Figs. 5, 6, 7, 11, 12 & 13).

Level 1 GO annotations between *Gambierdiscus* species. The GO annotations found at level 1 between the species of *Gambierdiscus* were similar, with the exception

of *G. australes* in several instances. For GOs assigned part of catalytic activity in molecular processes (Fig. 10) as well as both the metobolic and cellular processes in the biological processes (Fig. 8), *G. australes* was underrepresented. Within the molecular processes (Fig. 10), the most common annotation was for catalytic activity, followed by binding then transporter activities. Molecular carrier activity was only registered for *G. australes* and *G. carpenteri* with 1 annotation each. For GO annotations within the cellular processes (9), the most common match was to cell parts followed by protein containing complexes then organelle parts. Only *G. carpenteri* and *G. polynesiensis* had one annotation each for cell junction activity. The highest number of GOs within biological processes matched to cellular processes (Fig. 8), closely followed by metabolic processes then biological regulation and localization. The lease represented biological GO annotation was related to growth with only one annotation for *G. holmesii* and *G. polynesiensis*.

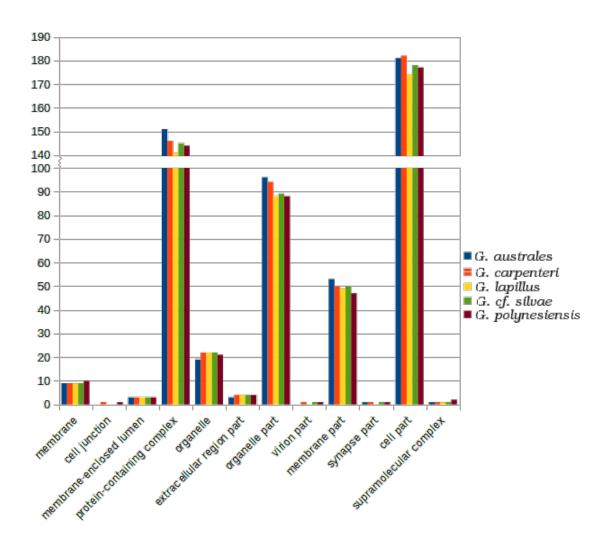


Figure 2: Summary of cellular GO annotations between *Gambierdiscus* species at GO-SUM level 1 from Suppl. table 3.

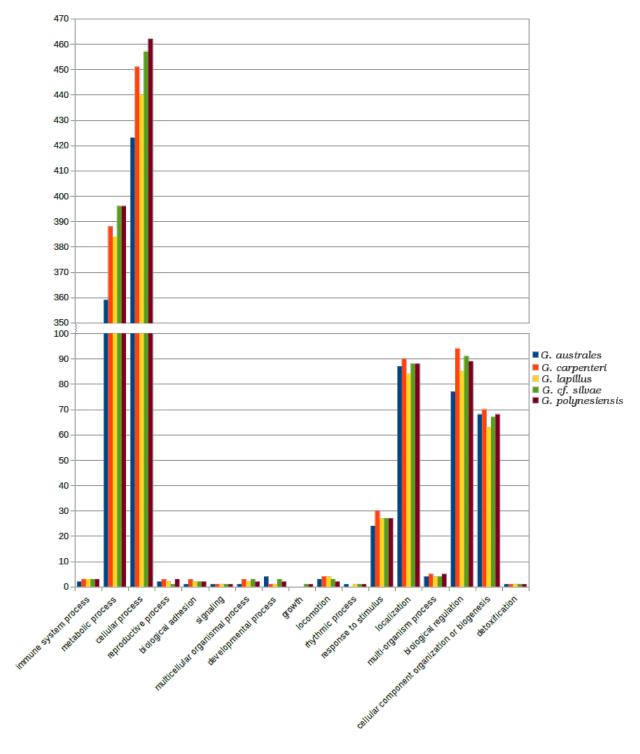


Figure 3: Summary of biological processes GO annotations between *Gambierdiscus* species at GOSUM level 1 from Suppl. table 3.

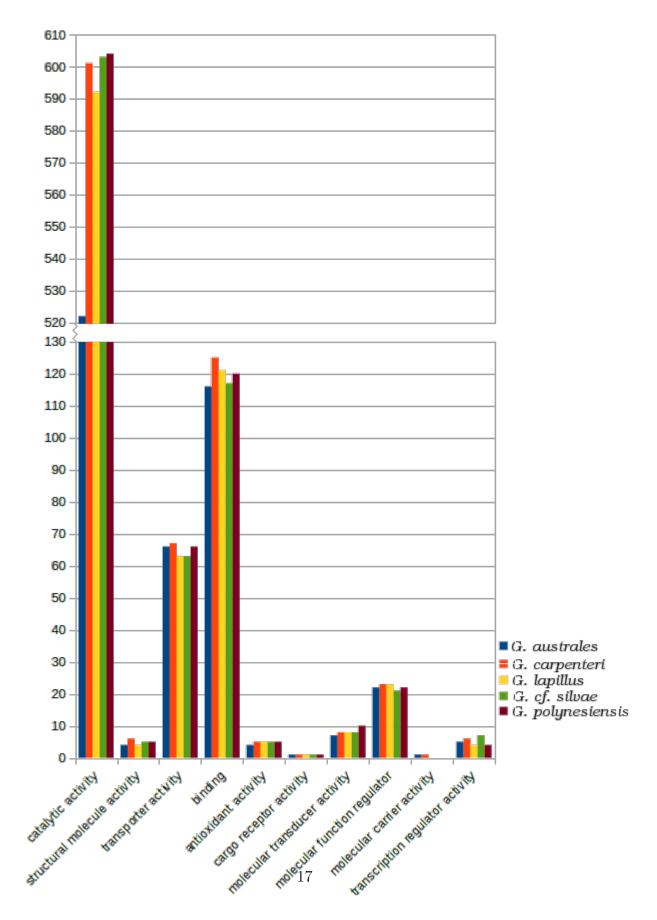


Figure 4: Summary of molecular GO annotations between *Gambierdiscus* species at GOSUM level 1 from Suppl. table 3.

Level 2 GO annotations between *Gambierdiscus* species. At level 2 of GO annotations, difference between species becomes more apparent. While inter-species variations across the molecular, cellular and biological processes (Figs. 11, 12 & 13) are apparent, consistently G. australes is underrepresented or absent across all three processes. Conversely, G. australes was the only species with a small number of GO annotations to nucleocytoplasmic carrier activity as well as general transcription initiation factor activity within the molecular processes, and anatomical structure morphogenisis as well as movement within environment as part of symbiotic interaction in the biological processes. G. holmesii had a much higher representation of GO terms matching sperm-egg recognition. The most common molecular process (Fig. 13) mapped to transferase activities, followed by hydrolase activity and oxidoreductase activity. For cellular processes (Fig. 12) the highest number of GOs was matched to intracellular parts, then intracellular organelle parts and membrane protein complexes. Organic substance metabolic processes, cellular metabolic processes and primary metabolic processes had the most GO annotation matches, in that order, for the biological processes group (Fig. 11).

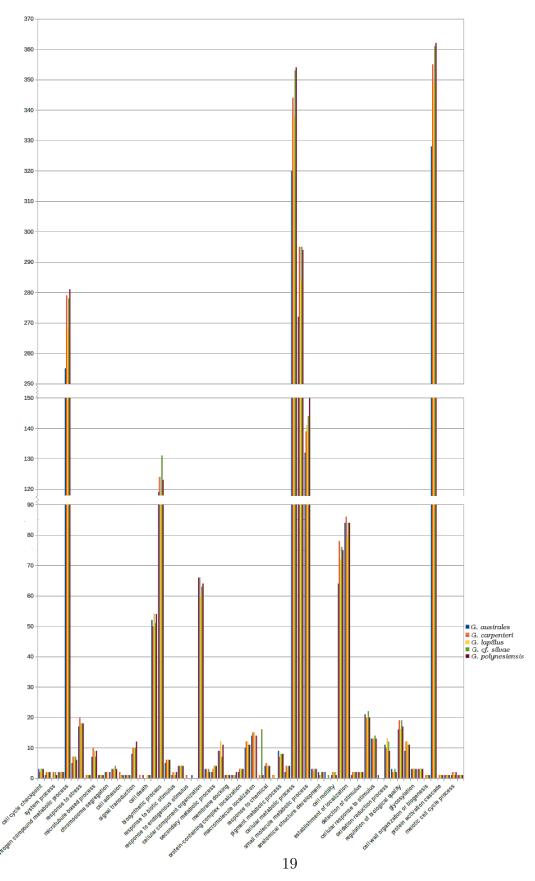


Figure 5: Summary of biological processes GO annotations between *Gambierdiscus* species at GOSUM level 2 from Suppl. table 4.

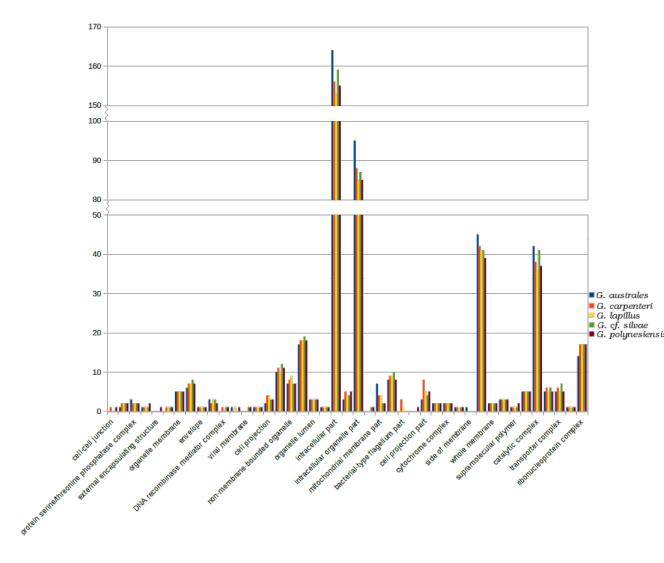


Figure 6: Summary of cellular GO annotations between *Gambierdiscus* species at GO-SUM level 2 from Suppl. table 4.

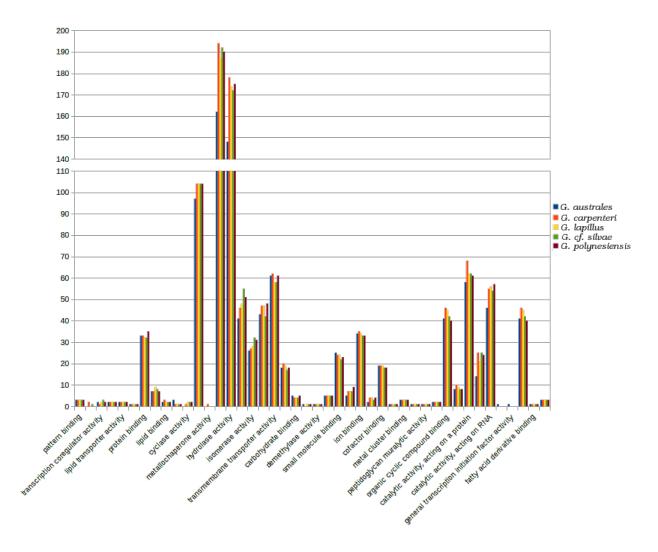


Figure 7: Summary of molecular GO annotations between *Gambierdiscus* species at GOSUM level 2 from Suppl. table 4.

Level 1 GO annotations for pan-transcriptomes. Similarity between the core-, softcore- and unique-transcriptomes was consistent across the biological, cellular and molecular processes groups. Predominantly the unique clusters had a higher represenation in each process with the exception for annotations matching to extracellular region parts and synpase parts within cellular processes (Fig. 9) as well as developmental processes within the biological processes (Fig. 8). GO annotations most commonly matched to catalytic activity then binding and transporter activities in the molecular processes (Fig. 10). Within the cellular processes, annotations predominantly matched to cellular parts, followed by protein-containing complexes and organelle parts (Fig. 8). For biological processes, the prevalent GO annotations matched to cellular processes, metabolic processes and localization (Fig. 8).

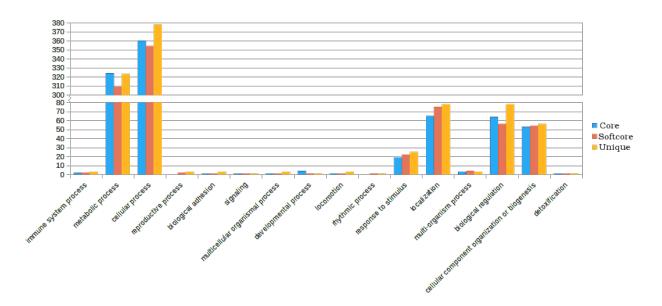


Figure 8: Summary of biological processes GO annotations between core, softcore and unique clusters at GOSUM level 1.

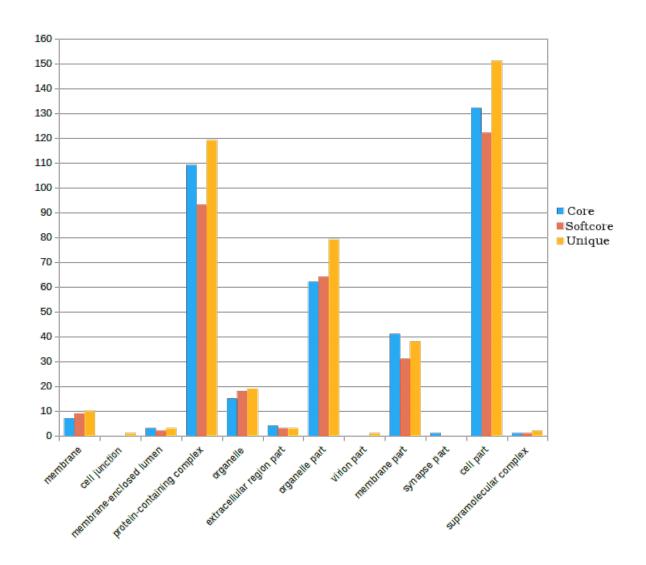


Figure 9: Summary of cellular GO annotations between core, softcore and unique clusters at GOSUM level 1.

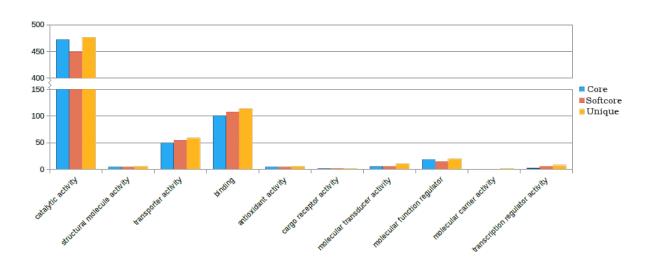


Figure 10: Summary of molecular GO annotations between core, softcore and unique clusters at GOSUM level 1.

Level 2 GO annotations for pan-transcriptomes. While differences between the biological, cellular and molecular processes were more distinctive at level 2, with the most common pattern among all three groupings of the core and unique clusters closely matching the number of GO terms with one or the other dominant, and the softcore clusters as less prevalent (Figs. 11, 12 & 13). Only the unique clusters had annotations matching to DNA binding transcription factor activity, metallochaperone activity and water binding in the molecular processes while the most common GO annotations for the pan-transcriptome matched to transferase, hydrolase and oxidoreductase activities in descending order (Fig. 13). Within the cellular processes, most annotations matched to intracellular parts, followed by intracellular organelle parts then membrane protein complexes (Fig. 12). Annotations solely from unique clusters were from cell-cell junction complexes, a viral membrane, contractile fibre parts, bacterila-type flagellum and an external encapsulating structure part. The biological processes annotations most commonly matched to organic substance metabolic processes, cellular metabolic processes and primary metabolic processes in descending order (Fig. 11). Unique clusters were the only representatives for system processes, immune response, cell adhesion, cell death, sperm-egg recognition, cell motility and a protein activation cascade.

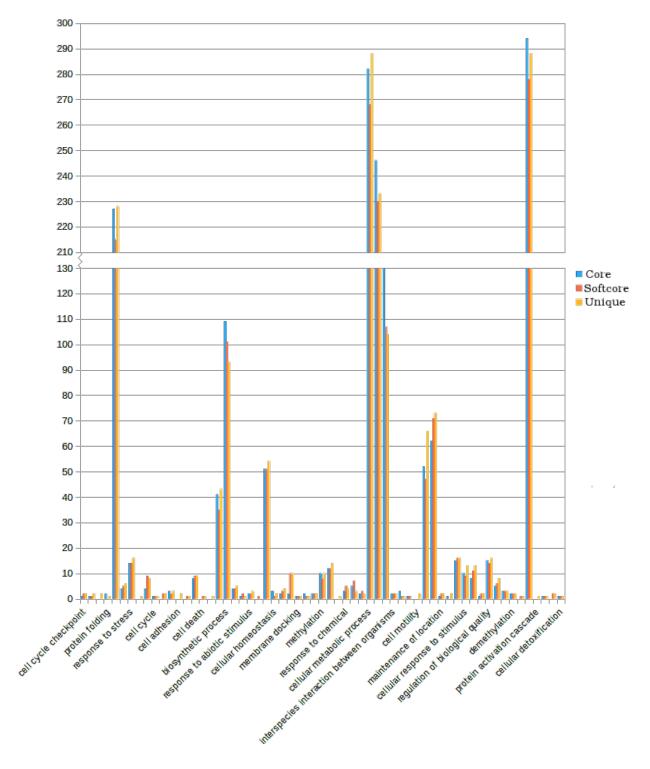


Figure 11: Summary of biological processes GO annotations between core, softcore and unique clusters at GOSUM level 2.

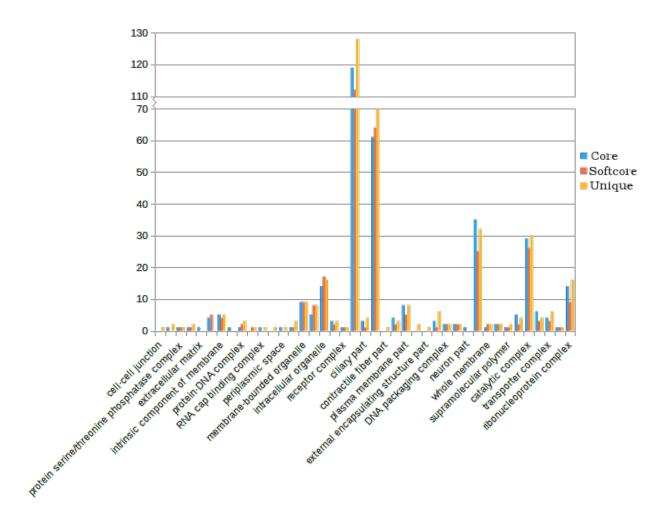


Figure 12: Summary of cellular GO annotations between core, softcore and unique clusters at GOSUM level 2.

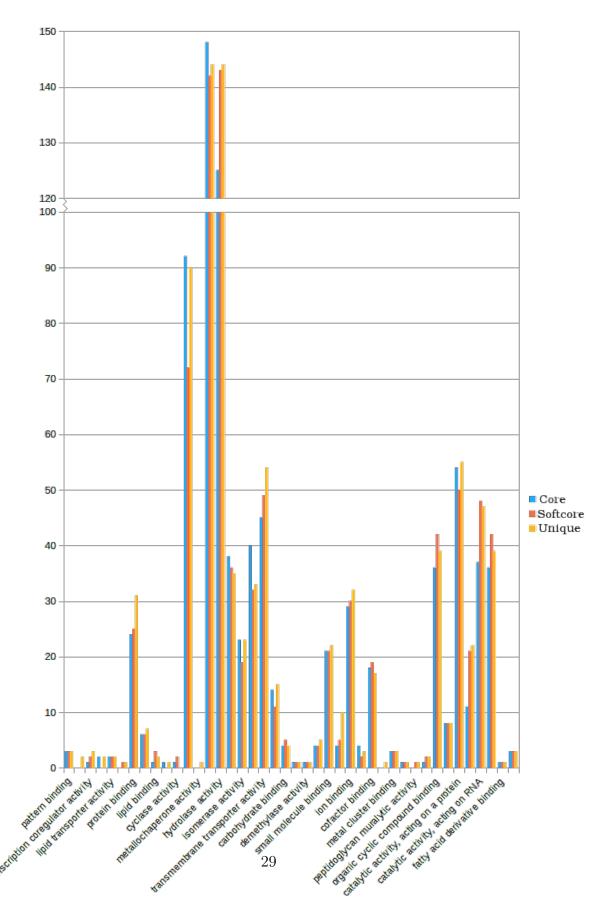


Figure 13: Summary of molecular GO annotations between core, softcore and unique clusters at GOSUM level 2.

### 4.2 Keto synthase active domain search

A total of 850 contigs were identified with KS domains which assembled into 314 clusters (Fig. 14). Nine clusters contained more than 10 contigs, with the highest number of 130 contigs from all species. 9 clusters contained 10 contigs or more, of which only two did not contain all the taxa examined. 57 of the 314 clusters contained contigs from multiple species, so 81.8 % of KS clusters were species specific while 78.7 % contained only a single contig (Fig. 14). The non-ciguatoxic G. carpenteri was absent from 73.6 % of the clusters. Of the clusters without G. carpenteri, none contained all four other species. However one cluster contained G. lapillus, G. polynesiensis and G. cf. silvae with equally represented transcript numbers. Four contigs contained G. polynesiensis and G. cf. silvae only, one of which had a higher contig representation of G. polynesiensis than G. cf. silvae. G. polynesiensis was the only representative species in 71 clusters, of which three clusters contained 2 contigs and one cluster contained 3 contigs. G. cf. silvae was representative as the only species in 23 clusters, one of which contained 3 contigs while the other clusters contained single contigs. G. australes, G. carpenteri and G. lapillus were the solo representatives of 81, 39 & 35 KS clusters respectively.

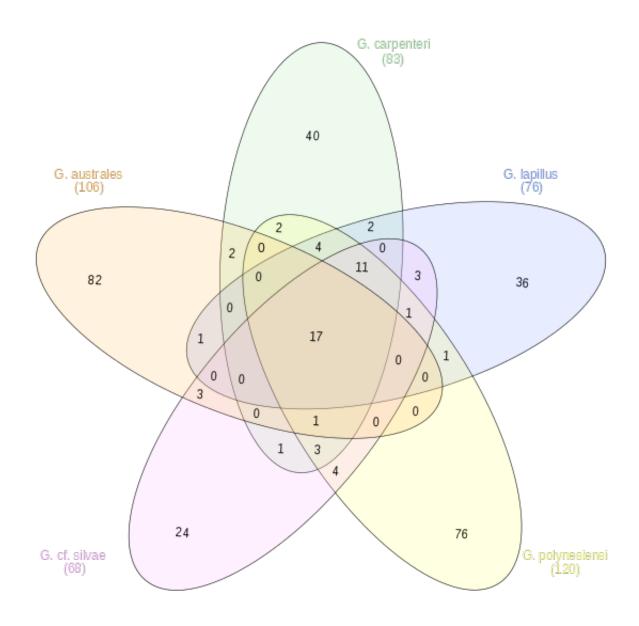


Figure 14: Venn diagram of species in KS clusters.

# 5 Discussion

Comparing five *Gambierdiscus* species revealed a core, soft-core and unique fraction between the transcriptomes. Further, differences between species with different toxin production characteristics were observed.

# 5.1 Core, soft-core and unique genes

The number of contigs and predicted peptides from this study was markedly variable between G. australies, from the MMETSP dataset, in comparison to the transcriptome assemblies generated in **Chapter 4**. To accommodate for the low number of contigs from G. australes, the soft-core spanned 4 of the 5 taxa. Noticeably, G. australes was absent from 86 % of the soft-core dataset which indicates that a large proportion of the soft-core is likely part of the G ambierdiscus pan-transcriptome core which was not captured in the G. australes sequencing. This is a case where the relevance of a reference pan-transcriptome for sequencing efforts becomes evident.

Tim up to here, the rest is mostly based in the whole seq dataset and ideas on what to possibly look at

To go here:

- overall summary of study
- core and pan more likely to be accurate without being axenic unless same contamination vs removing bact LCA
- spliced leader sites really low. potentially interesting the two highest ones are from same phylogenetic clade, while the other three are representatives from the other two main clades. Also poly and silvae are from separate seq runs, so not an artefact from that front.
- G. australes seq is quite bad in comparison as can be seen in the GOSUM figs and the comparative number of contigs, predicted proteins and softcore clusters
- **Tim** not sure if I can do something like Fig 2 only 5 isolates to put in, and I think I need to look at that again with more sleep to work out what's going on and if I

could transfer the concept., there are over 200,000 pan-tran clusters, I don't think I can work out whether they are genophyletic or monophyletic for that many

#### 5.2 dinoSL

-differences between transcriptomes... either seq metod related, or taxa relate. I think silvae and poly had the most, which are from the same sub clade? - super low number of dinoSL found in libraries, not representative of all the transcripts [9] and they cite [2] as similar, but incompatible with findings by [44].. check zhang 2007 is it a detection thing, or genus/species specific differences

# core Gambierdiscus transcriptome

[25] comprehensive index of genes in K. brevis to compare to as well as functional summaries

#### discuss common & different functions found

Koid 14 pan-transcriptome of 4 prymnesiophyte algae. Compare functional findings (KOG vs. this) and contigs as well as predicted protein coding regions are just a fraction of the ones here. eg.30,000-56,000 contigs vs. lowest for in this study is 148,972. Other study transcriptomes are part of MMETSP, but even australes here is over 100,000 contigs which is from the same study so more likely it's a Gambi thing rather than a seq thing. same with australes and Koid for peptides predicted, almost double. Way higher for other gambis.

#### Expression of genes involved in polyketide production

- discuss if different gene sets were expressed between toxic and non-toxic strains (ie. not carp)
- discuss KS containing contigs per species plus distribution and number of contigs in KS clusters

- point at Venn diagram intersections that could be of interest for further investigation for both MTX and CTX
- discuss KS conserved region phylogeny
- **Tim** I'm not sure we know enough about these pathways to do something like fig 5

#### Bacterial association with host

- really depends what the basta results are and if anything interesting is found
- 'fundamental shift' in transcript expression observed in A. tamarense based on bacterial presence, much higher than N or P depletion [28]

#### discuss usefulness for future studies

- Usefullness of core transcriptome for RNA sequencing studies
- Investigate poly only KS clusters or clusters with high number of poly reps

#### discuss potential short comings

- from different seq runs and methods and seq depth may vary, especially G. australes
- intra-speces variation so one isolate per species may not be representative
- unknown if processes other than PKS play a role in toxin production

# Conclusion

# Supplementary

- need to add australes

Table 3: GO terms and number of contigs per species at GO ontology level 1.

GO aces-	GO terms	G. carpen-	G. lapillus	G. polyne-	G. cf. sil-	
sion		l teri		siensis	vae	
	Bi	ological proce	sses			
GO:0002376	immune system pro-	3	3	3	3	
	cess					
GO:0008152	metabolic process	388	384	396	396	
GO:0009987	cellular process	451	440	457	462	
GO:0022414	reproductive process	3	2	1	3	
GO:0022610	biological adhesion	3	2	2	2	
GO:0023052	signaling	1	1	1	1	
GO:0032501	multicellular organis-	3	2	3	2	
	mal process					
GO:0032502	developmental process	1	1	3	2	
GO:0040007	growth	0	0	1	1	
GO:0040011	locomotion	4	4	3	2	
GO:0048511	rhythmic process	0	1	1	1	
GO:0050896	response to stimulus	30	27	27	27	
GO:0051179	localization	90	84	88	88	
GO:0051704	multi-organism pro-	5	4	4	5	
	cess					
GO:0065007	biological regulation	94	85	91	89	
GO:0071840	cellular component or-	70	63	67	68	
	ganization/biogenesis					
GO:0098754	detoxification	1	1	1	1	
Cellular components						
GO:0016020	membrane	9	9	9	10	
GO:0030054	cell junction	1	0	0	1	
GO:0031974	membrane-enclosed	3	3	3	3	
	lumen					

GO:0032991	protein-containing	146	141	145	144		
33.002201	complex	110					
GO:0043226	organelle	22	22	22	21		
GO:0044421	extracellular region	4	4	4	4		
G. G. G. G. G. T. T. Z. T.	part						
GO:0044422	organelle part	94	88	89	88		
GO:0044423	virion part	1	0	1	1		
GO:0044425	membrane part	50	49	50	47		
GO:0044456	synapse part	1	0	1	1		
GO:0044464	cell part	182	174	178	177		
GO:0099080	supramolecular com-	1	1	1	2		
40.000000	plex			1			
Molecular function							
GO:0003824	catalytic activity	601	592	603	604		
GO:0005198	structural molecule	6	4	5	5		
GO:0003130	activity	U	<b>1</b>				
GO:0005215	transporter activity	67	63	63	66		
GO:0005488	binding	125	121	117	120		
GO:0016209	antioxidant activity	5	5	5	5		
GO:0038024	cargo receptor activity	1	1	1	1		
GO:0060089	molecular transducer	8	8	8	10		
GO:0000089	activity	0	0	0	10		
CO.0002772	molecular function	23	23	21	22		
GO:0098772		40	20	21			
CO.0140104	regulator molecular carrier ac-	1	0	0	0		
GO:0140104		1	U	0	U		
00.0140110	tivity	C	1	7	1		
GO:0140110	transcription regula-	6	4	7	4		
	tor activity						

Table 4: GO terms and number of contigs per species at GO ontology level 2, child terms of Table 3.

GO aces-	GO terms	G. carpen-	G. lapillus	G. polyne-	G. cf. sil-		
sion		l teri		siensis	vae		
Biological processes							
GO:0000075	cell cycle checkpoint	2	3	3	3		
GO:0002252	immune effector pro-	2	2	2	2		
	cess						
GO:0003008	system process	2	1	2	1		
GO:0006457	protein folding	2	2	2	2		
GO:0006807	nitrogen compound	279	268	278	281		
	metabolic process						
GO:0006928	movement of cell or	7	7	7	6		
	subcellular compo-						
	nent						
GO:0006950	response to stress	20	18	18	18		
GO:0006955	immune response	1	1	1	1		
GO:0007017	microtubule-based	10	9	7	9		
	process						
GO:0007049	cell cycle	1	1	1	1		
GO:0007059	chromosome segrega-	2	2	0	2		
	tion						
GO:0007154	cell communication	3	3	4	3		
GO:0007155	cell adhesion	2	1	1	1		
GO:0007163	establishment or	1	0	1	1		
	maintenance of cell						
	polarity						
GO:0007165	signal transduction	10	10	10	12		
GO:0008037	cell recognition	1	0	0	1		
GO:0008219	cell death	0	1	1	1		
GO:0009056	catabolic process	50	54	51	54		

GO:0009058	biosynthetic process	124	119	131	123
GO:0009605	response to external	6	6	6	6
	stimulus				
GO:0009607	response to biotic	2	2	1	2
	stimulus				
GO:0009628	response to abiotic	4	4	4	4
	stimulus				
GO:0009719	response to endoge-	1	0	0	0
	nous stimulus				
GO:0016043	cellular component or-	66	60	63	64
	ganization				
GO:0019725	cellular homeostasis	3	2	3	2
GO:0019748	secondary metabolic	3	4	4	4
	process				
GO:0022402	cell cycle process	9	12	7	11
GO:0022406	membrane docking	1	1	1	1
GO:0030029	actin filament-based	1	0	1	2
	process				
GO:0031503	protein-containing	3	3	3	3
	complex localization				
GO:0032259	methylation	12	12	11	11
GO:0033036	macromolecule local-	15	15	0	14
	ization				
GO:0035036	sperm-egg recognition	1	0	16	1
GO:0042221	response to chemical	5	4	4	4
GO:0042330	taxis	1	1	0	0
GO:0042440	pigment metabolic	7	8	8	8
	process				
GO:0044085	cellular component	4	3	4	4
	biogenesis				

GO:0044237	cellular metabolic pro-	344	338	353	354
GO 0044222	cess	205	20.4	205	20.4
GO:0044238	primary metabolic	295	284	295	294
	process				
GO:0044281	small molecule	139	141	144	150
	metabolic process				
GO:0044419	interspecies inter-	3	2	3	3
	action between				
	organisms				
GO:0048856	anatomical structure	1	1	2	2
	development				
GO:0048869	cellular developmental	0	0	1	
	process				
GO:0048870	cell motility	2	2	2	1
GO:0050789	regulation of biologi-	78	72	76	75
	cal process				
GO:0051234	establishment of local-	86	79	84	84
	ization				
GO:0051235	maintenance of loca-	2	2	2	2
	tion				
GO:0051606	detection of stimulus	2	2	2	2
GO:0051641	cellular localization	20	20	22	20
GO:0051716	cellular response to	13	13	14	13
	stimulus				
GO:0055114	oxidation-reduction	10	13	12	9
	process				
GO:0061919	process utilizing au-	2	2	3	2
	tophagic mechanism				
GO:0065008	regulation of biologi-	19	16	19	17
	cal quality				
	1 /				

GO:0065009	regulation of molecu-	12	12	11	11	
	lar function					
GO:0070085	glycosylation	3	3	3	3	
GO:0070988	demethylation	3	3	3	3	
GO:0071554	cell wall organization	1	1	1	1	
	or biogenesis					
GO:0071704	organic substance	355	349	361	362	
	metabolic process					
GO:0072376	protein activation cas-	1	1	1	1	
	cade					
GO:0140029	exocytic process	1	1	1	1	
GO:1903046	meiotic cell cycle pro-	2	2	1	2	
	cess					
GO:1990748	cellular detoxification	1	1	1	1	
	Cellular components					
GO:0005911	cell-cell junction	1	0	0	1	
GO:0005929	cilium	2	2	2	2	
GO:0008287	protein ser-	2	2	2	2	
	ine/threonine phos-					
	phatase complex					
GO:0019867	outer membrane	1	1	1	2	
GO:0030312	external encapsulat-	0	0	0	1	
	ing structure					
GO:0031012	extracellular matrix	1	1	1	1	
GO:0031090	organelle membrane	5	5	5	5	
GO:0031224	intrinsic component of	7	7	8	7	
	membrane					
GO:0031975	envelope	1	1	1	1	
GO:0032993	protein-DNA complex	2	3	3	2	
GO:0033061	DNA recombinase me-	1	0	1	1	
	diator complex					

GO:0034518	RNA cap binding	0	1	0	1
	complex				
GO:0036338	viral membrane	0	0	1	1
GO:0042597	periplasmic space	1	1	1	1
GO:0042995	cell projection	4	4	3	3
GO:0043227	membrane-bounded	11	10	12	11
	organelle				
GO:0043228	non-membrane-	8	9	7	7
	bounded organelle				
GO:0043229	intracellular organelle	18	18	19	18
GO:0043233	organelle lumen	3	3	3	3
GO:0043235	receptor complex	1	1	1	1
GO:0044424	intracellular part	156	153	159	155
GO:0044441	ciliary part	5	4	4	5
GO:0044446	intracellular organelle	88	85	87	85
	part				
GO:0044449	contractile fiber part	0	0	1	1
GO:0044455	mitochondrial mem-	4	4	2	2
	brane part				
GO:0044459	plasma membrane	9	9	10	8
	part				
GO:0044461	bacterial-type flagel-	3	1	0	0
	lum part				
GO:0044462	external encapsulat-	0	0	0	1
	ing structure part				
GO:0044463	cell projection part	8	5	4	5
GO:0044815	DNA packaging com-	2	2	2	2
	plex				
GO:0070069	cytochrome complex	2	2	2	2
GO:0097458	neuron part	1	0	1	1

GO:0098796	membrane protein complex	42	41	41	39		
GO:0098805	whole membrane	2	2	2	2		
GO:0099023	tethering complex	3	3	3	3		
GO:0099081	supramolecular poly-	1	1	1	2		
GO:0099001	mer	1	1	1	2		
GO:0120114	Sm-like protein family	5	5	5	5		
00.0120114	complex						
GO:1902494	catalytic complex	38	36	41	37		
GO:1990204	oxidoreductase com-	6	5	6	5		
	plex						
GO:1990351	transporter complex	6	5	7	5		
GO:1990391	DNA repair complex	1	1	1	1		
GO:1990904	ribonucleoprotein	17	17	17	17		
	complex						
Molecular function							
GO:0001871	pattern binding	3	3	3	3		
GO:0003700	DNA-binding tran-	2	0	1	0		
	scription factor						
	activity						
GO:0003712	transcription coregu-	1	2	3	2		
	lator activity						
GO:0004133	glycogen debranching	2	2	2	2		
	enzyme activity						
GO:0005319	lipid transporter ac-	2	2	2	2		
	tivity						
GO:0005326	neurotransmitter	1	1	1	1		
	transporter activity						
GO:0005515	protein binding	33	32	32	35		
GO:0008144	drug binding	7	9	8	7		
GO:0008289	lipid binding	3	2	2	2		

GO:0008565	protein transporter	1	1	1	1
	activity				
GO:0009975	cyclase activity	1	2	2	2
GO:0016491	oxidoreductase activ-	104	104	104	104
	ity				
GO:0016530	metallochaperone ac-	1	0	0	0
	tivity				
GO:0016740	transferase activity	194	187	192	190
GO:0016787	hydrolase activity	178	174	172	175
GO:0016829	lyase activity	46	48	55	51
GO:0016853	isomerase activity	27	28	32	31
GO:0016874	ligase activity	47	47	42	48
GO:0022857	transmembrane trans-	62	58	58	61
	porter activity				
GO:0030234	enzyme regulator ac-	20	19	17	18
	tivity				
GO:0030246	carbohydrate binding	4	4	4	5
GO:0030545	receptor regulator ac-	0	1	1	1
	tivity				
GO:0032451	demethylase activity	1	1	1	1
GO:0033218	amide binding	5	5	5	5
GO:0036094	small molecule bind-	24	24	22	23
	ing				
GO:0038023	signaling receptor ac-	7	7	7	9
	tivity				
GO:0043167	ion binding	35	34	33	33
GO:0044877	protein-containing	4	4	3	4
	complex binding				
GO:0048037	cofactor binding	19	19	18	18
GO:0050824	water binding	1	1	1	1
GO:0051540	metal cluster binding	3	3	3	3

GO:0060090	molecular adaptor ac-	1	1	1	1
	tivity				
GO:0061783	peptidoglycan mura-	1	1	1	1
	lytic activity				
GO:0072341	modified amino acid	2	2	2	2
	binding				
GO:0097159	organic cyclic com-	46	45	42	40
	pound binding				
GO:0097367	carbohydrate deriva-	10	9	8	8
	tive binding				
GO:0140096	catalytic activity, act-	68	62	62	61
	ing on a protein				
GO:0140097	catalytic activity, act-	25	21	25	24
	ing on DNA				
GO:0140098	catalytic activity, act-	55	56	54	57
	ing on RNA				
GO:1901363	heterocyclic com-	46	45	42	40
	pound binding				
GO:1901567	fatty acid derivative	1	1	1	1
	binding				
GO:1901681	sulfur compound	3	3	3	3
	binding				

Table 5: GO terms and number of contigs found in core, softcore and pan-transcriptome of Gambierdiscus at GO ontology level 1.

	GO	aces-	GO terms		Core	Softcore	Pan	
si	on							
	Biological processes							

GO:0002376	immune system pro-	2	2	3
	cess			
GO:0008152	metabolic process	324	309	323
GO:0009987	cellular process	360	354	378
GO:0022414	reproductive process	0	2	3
GO:0022610	biological adhesion	1	1	3
GO:0023052	signaling	1	1	1
GO:0032501	multicellular organis-	1	1	3
	mal process			
GO:0032502	developmental process	4	1	1
GO:0040011	locomotion	1	1	3
GO:0048511	rhythmic process	0	1	1
GO:0050896	response to stimulus	19	22	25
GO:0051179	localization	65	75	78
GO:0051704	multi-organism pro-	3	4	3
	cess			
GO:0065007	biological regulation	64	56	78
GO:0071840	cellular component or-	53	54	56
	ganization or biogene-			
	sis			
GO:0098754	detoxification	1	1	1
	Cellular o	components		
GO:0016020	membrane	7	9	10
GO:0030054	cell junction	0	0	1
GO:0031974	membrane-enclosed	3	2	3
	lumen			
GO:0032991	protein-containing	109	93	119
	complex			
GO:0043226	organelle	15	18	19
GO:0044421	extracellular region	4	3	3
	part			

GO:0044422	organelle part	62	64	79
GO:0044423	virion part	0	0	1
GO:0044425	membrane part	41	31	38
GO:0044456	synapse part	1	0	0
GO:0044464	cell part	132	122	151
GO:0099080	supramolecular com-	1	1	2
	plex			
	Molecula	r function		
GO:0003824	catalytic activity	472	449	476
GO:0005198	structural molecule	4	4	5
	activity			
GO:0005215	transporter activity	49	54	58
GO:0005488	binding	100	107	113
GO:0016209	antioxidant activity	4	4	5
GO:0038024	cargo receptor activity	1	1	1
GO:0060089	molecular transducer	5	5	10
	activity			
GO:0098772	molecular function	18	14	19
	regulator			
GO:0140104	molecular carrier ac-	0	0	1
	tivity			
GO:0140110	transcription regula-	2	5	7
	tor activity			

Table 6: GO terms and number of contigs found in core, softcore and pan-transcriptome of *Gambierdiscus* at GO ontology level 2, childer to Table 5.

GO aces-	GO terms	Core	Softcore	Pan				
sion								
	Biological processes							

GO:0000075	cell cycle checkpoint	1	2	2
GO:0002252	immune effector pro-	1	1	2
	cess			
GO:0003008	system process	0	0	2
GO:0006457	protein folding	2	0	1
GO:0006807	nitrogen compound	227	215	228
	metabolic process			
GO:0006928	movement of cell or	4	5	6
	subcellular compo-			
	nent			
GO:0006950	response to stress	14	14	16
GO:0006955	immune response	0	0	1
GO:0007017	microtubule-based	4	9	8
	process			
GO:0007049	cell cycle	1	1	1
GO:0007059	chromosome segrega-	0	2	2
	tion			
GO:0007154	cell communication	3	2	3
GO:0007155	cell adhesion	0	0	2
GO:0007163	establishment or	0	1	1
	maintenance of cell			
	polarity			
GO:0007165	signal transduction	8	9	9
GO:0008037	cell death	0	1	1
GO:0008219	cell death	0	0	1
GO:0009056	catabolic process	41	35	43
GO:0009058	biosynthetic process	109	101	93
GO:0009605	response to external	4	4	5
	stimulus			
GO:0009607	response to biotic	1	2	1
	stimulus			

GO:0009628	response to abiotic stimulus	2	2	3
GO:0009719	response to endogenous stimulus	0	1	0
GO:0016043	cellular component organization	51	51	54
GO:0019725	cellular homeostasis	3	1	2
GO:0019748	secondary metabolic process	2	3	4
GO:0022402	cell cycle process	2	10	10
GO:0022406	membrane docking	1	1	1
GO:0030029	actin filament-based process	2	1	1
GO:0031503	protein-containing complex localization	2	2	2
GO:0032259	methylation	10	8	10
GO:0033036	macromolecule localization	12	12	14
GO:0035036	sperm-egg recognition	0	0	1
GO:0042221	response to chemical	3	5	4
GO:0042440	pigment metabolic process	5	7	3
GO:0044085	cellular component biogenesis	2	3	2
GO:0044237	cellular metabolic process	282	268	288
GO:0044238	primary metabolic process	246	230	233
GO:0044281	small molecule metabolic process	130	107	104

GO:0044419	interspecies inter-	2	2	2
	action between			
	organisms			
GO:0048856	anatomical structure	3	1	1
	development			
GO:0048869	cellular developmental	1	1	0
	process			
GO:0048870	cell motility	0	0	2
GO:0050789	regulation of biologi-	52	47	66
	cal process			
GO:0051234	establishment of local-	62	71	73
	ization			
GO:0051235	maintenance of loca-	1	2	2
	tion			
GO:0051606	detection of stimulus	1	0	2
GO:0051641	cellular localization	15	16	16
GO:0051716	cellular response to	10	9	13
	stimulus			
GO:0055114	oxidation-reduction	8	11	13
	process			
GO:0061919	process utilizing au-	1	2	2
	tophagic mechanism			
GO:0065008	regulation of biologi-	15	14	16
	cal quality			
GO:0065009	regulation of molecu-	5	6	8
	lar function			
GO:0070085	glycosylation	3	3	3
GO:0070988	demethylation	2	2	2
GO:0071554	cell wall organization	0	1	1
	or biogenesis			

GO:0071704	organic substance metabolic process	294	278	288
GO:0072376	protein activation cascade	0	0	1
GO:0140029	exocytic process	1	1	1
GO:1903046	meiotic cell cycle pro-	0	2	2
	cess			
GO:1990748	cellular detoxification	1	1	1
	Cellular o	components		
GO:0005911	cell-cell junction	0	0	1
GO:0005929	cilium	1	0	2
GO:0008287	protein ser-	1	1	1
	ine/threonine phos-			
	phatase complex			
GO:0019867	outer membrane	1	1	2
GO:0031090	extracellular matrix	1	0	0
GO:0031090	organelle membrane	4	5	0
GO:0031224	intrinsic component of membrane	5	4	5
GO:0031975	envelope	1	0	0
GO:0032993	protein-DNA complex	1	2	3
GO:0033061	DNA recombinase mediator complex	0	1	1
GO:0034518	RNA cap binding complex	1	0	1
GO:0036338	viral membrane	0	0	1
GO:0042597	periplasmic space	1	0	1
GO:0042995	cell projection	1	1	3
GO:0043227	membrane-bounded organelle	9	9	9

GO:0043228	non-membrane-	5	8	8
	bounded organelle			
GO:0043229	intracellular organelle	14	17	16
GO:0043233	organelle lumen	3	2	3
GO:0043235	receptor complex	1	1	1
GO:0044424	intracellular part	119	112	128
GO:0044441	ciliary part	3	1	4
GO:0044446	intracellular organelle part	61	64	74
GO:0044449	contractile fiber part	0	0	1
GO:0044455	mitochondrial mem- brane part	4	2	3
GO:0044459	plasma membrane part	8	5	8
GO:0044461	bacterial-type flagel- lum part	0	0	2
GO:0044462	external encapsulat- ing structure part	0	0	1
GO:0044463	cell projection part	3	1	6
GO:0044815	DNA packaging complex	2	2	2
GO:0070069	cytochrome complex	2	2	2
GO:0097458	neuron part	1	0	0
GO:0098796	membrane protein complex	35	25	32
GO:0098805	whole membrane	1	2	2
GO:0099023	tethering complex	2	2	2
GO:0099081	supramolecular poly- mer	1	1	2
GO:0120114	Sm-like protein family complex	5	2	4

GO:1902494	catalytic complex	29	26	30
GO:1990204	oxidoreductase com-	6	3	4
	plex			
GO:1990351	transporter complex	4	3	6
GO:1990391	DNA repair complex	1	1	1
GO:1990904	ribonucleoprotein	14	9	16
	complex			
	Molecula	r function		
GO:0001871	pattern binding	3	3	3
GO:0003700	DNA-binding tran-	0	0	2
	scription factor			
	activity			
GO:0003712	transcription coregu-	1	2	3
	lator activity			
GO:0004133	glycogen debranching	2	0	2
enzyme activity				
GO:0005319	lipid transporter ac-	2	2	2
	tivity			
GO:0005326	neurotransmitter	0	1	
	transporter activity			
GO:0005515	protein binding	24	25	31
GO:0008144	drug binding	6	6	7
GO:0008289	lipid binding	1	3	2
GO:0008565	protein transporter	1	0	1
	activity			
GO:0009975	cyclase activity	1	2	0
GO:0016491	oxidoreductase activ-	92	72	90
	ity			
GO:0016530	metallochaperone ac-	0	0	
	tivity			
GO:0016740	transferase activity	148	142	144

		I	I	
GO:0016787	hydrolase activity	125	143	144
GO:0016829	lyase activity	38	36	35
GO:0016853	isomerase activity	23	19	23
GO:0016874	ligase activity	40	32	33
GO:0022857	transmembrane trans-	45	49	54
	porter activity			
GO:0030234	enzyme regulator ac-	14	11	15
	tivity			
GO:0030246	carbohydrate binding	4	5	4
GO:0030545	receptor regulator ac-	1	1	1
	tivity			
GO:0032451	demethylase activity	1	1	1
GO:0033218	amide binding	4	4	5
GO:0036094	small molecule bind-	21	21	22
ing				
GO:0038023	signaling receptor ac-	4	5	10
	tivity			
GO:0043167	ion binding	29	30	32
GO:0044877	protein-containing	4	2	3
	complex binding			
GO:0048037	cofactor binding	18	19	17
GO:0050824	water binding	0	0	1
GO:0051540	metal cluster binding	3	3	3
GO:0060090	molecular adaptor ac-	1	1	1
	tivity			
GO:0061783	peptidoglycan mura-	0	1	1
	lytic activity			
GO:0072341	modified amino acid	1	2	2
	binding			
GO:0097159	organic cyclic com-	36	42	39
	pound binding			

GO:0097367	carbohydrate deriva-	8	8	8
	tive binding			
GO:0140096	catalytic activity, act-	54	50	55
	ing on a protein			
GO:0140097	catalytic activity, act-	11	21	22
	ing on DNA			
GO:0140098	catalytic activity, act-	37	48	47
	ing on RNA			
GO:1901363	heterocyclic com-	36	42	39
	pound binding			
GO:1901567	fatty acid derivative	1	1	1
	binding			
GO:1901681	sulfur compound	3	3	3
	binding			

Table 7: KS domains found per cluster and total number of contigs present.

Cluster	G. aus-	G. carpenteri	G. lapillus	G. polyne-	G. cf. sil-	Total
ID	trales			siensis	vae	contigs
988	6	40	29	24	31	130
8866	3	24	14	24	16	81
3681	7	14	16	9	12	58
1921	3	10	6	4	6	29
46550	3	4	1	8	5	21
215601	0	4	1	8	5	18
360	1	4	3	3	4	15
15645	4	2	0	4	1	11
132980	0	1	4	3	2	10
45086	1	3	1	1	3	9
78009	0	2	2	3	2	9

38915	2	2	2	1	2	9
109763	0	2	0	5	1	8
37859	2	2	1	2	1	8
24847	1	1	1	3	2	8
162333	0	2	2	2	1	7
52333	1	2	1	1	1	6
136782	0	1	2	1	2	6
301971	0	0	2	2	2	6
152898	0	3	1	1	0	5
117472	0	2	1	1	1	5
196360	0	2	1	1	1	5
145445	0	1	1	2	1	5
131919	0	1	0	1	3	5
59207	1	1	1	1	1	5
31669	1	1	1	1	1	5
55678	1	1	1	1	1	5
40462	1	1	1	1	1	5
46899	1	1	1	1	1	5
37886	1	1	1	1	1	5
475329	0	0	0	4	1	5
162320_UTS	MER9A3_Ga	mBierdiscus-	0	1	0	4
carpenteri_I	N15967_c2_g1	_i2.p1.faa				
21082_MME	T\$P0766_Gar	n <b>b</b> ierdiscus-	0	2	1	4
australes_Dl	N32692_c0_g1_	i1.p1.faa				
195242_UTS	MER9A3_Ga	mbierdiscus-	1	1	1	4
carpenteri_I	N17326_c2_g5	_i1.p1.faa				
83891_UTSI	M <b>E</b> R9A3_Gan	b <b>i</b> erdiscus-	1	1	1	4
carpenteri_I	N13035_c1_g4	_i1.p1.faa				
99486_UTSI	M <b>E</b> R9A3_Gam	b <b>i</b> erdiscus-	1	1	1	4
carpenteri_I	N13588_c0_g3	Li1.p1.faa				

,	$328911\_\mathrm{HG4}\_\mathbf{G}$ ambierdisc	u <b>©</b>	1	3	0	4
]	lapillus_DN41464_c0_g1_i1	.p1.faa				
(	$643864\_{ m HG5}\_{ m m{G}}$ ambierdisc	u <b>©</b>	0	0	4	4
	silvae_DN47931_c1_g3_i1.p	2.faa				
-	186957_UTSMER9A3_Gar	mbierdiscus-	1	1	0	3
(	carpenteri_DN16979_c3_g3	_i1.p1.faa				
-	193820_UTS <b>M</b> ER9A3_Ga	mbierdiscus-	1	1	0	3
	carpenteri_DN17268_c1_g8	3_i4.p1.faa				
-	147284_UTS <b>M</b> ER9A3_Gar	mbierdiscus-	1	1	0	3
(	carpenteri_DN15408_c1_g3	3_i2.p1.faa				
	116539_UTS <b>M</b> ER9A3_Ga	mbierdiscus-	2	0	0	3
(	carpenteri_DN14227_c2_g1	_i4.p1.faa				
4	242595_UTS <mark>M</mark> ER9A3_Ga	mbierdiscus-	2	0	0	3
(	carpenteri_DN9176_c0_g1_	i3.p1.faa				
!	524928_CG150Gambierdis	c10s-	0	3	0	3
]	polynesiensis_DN43543_c1	_g1_i1.p1.faa				
	1040_MMETSP0766_Gam	b <b>0</b> erdiscus-	0	0	2	3
í	australes_DN11947_c0_g1_	i1.p1.faa				
,	38402_MME <b>T2</b> \$P0766_Gar	n $oldsymbol{b}$ ierdiscus-	1	0	0	3
-	australes_DN41494_c1_g1_					
-	154624_UTS <mark>ME</mark> R9A3_Gar	m <b>2</b> bierdiscus-	0	0	0	2
	carpenteri_DN15679_c0_g6	i1.p1.faa				
	63665_UTSMER9A3_Gam		0	0	0	2
	carpenteri_DN10182_c0_g1	_i2.p1.faa				
4	205876_UTS <mark>M</mark> ER9A3_Ga	m2bierdiscus-	0	0	0	2
-	carpenteri_DN17803_c0_g4					
	224239_UTS <mark>M</mark> ER9A3_Gar		0	0	0	2
-	carpenteri_DN18618_c3_g6					
	196786_UTS <mark>M</mark> ER9A3_Ga		0	1	0	2
	$carpenteri_DN17387_c2_g2$	Li1.p1.faa				

131133_UTS <b>M</b> ER9A3_Ga	mbierdiscus-	0	0	1	2
carpenteri_DN14782_c2_g	_i3.p1.faa				
19133_MMETSP0766_Ga	nbierdiscus-	0	0	0	2
$australes_DN30780_c0_g2_s$	i1.p1.faa				
37007_MMETSP0766_Gar	nbierdiscus-	0	0	0	2
$australes_DN41205_c1_g7_c$	i1.p1.faa				
$424979$ _CG1 $50$ Gambierdis	c10s-	0	2	0	2
polynesiensis_DN34166_c0	_g9_i1.p1.faa				
$358554$ _CG1 $50$ Gambierdis	c10s-	0	2	0	2
polynesiensis_DN15070_c0	_g1_i1.p2.faa				
$408901$ _CG1 $50$ Gambierdis	c10s-	0	2	0	2
polynesiensis_DN32288_c2	_g1_i1.p1.faa				
479997_CG150Gambierdis	c10s-	0	1	1	2
polynesiensis_DN39607_c0	_g2_i1.p1.faa				
485470_CG150Gambierdis	c10s-	0	1	1	2
polynesiensis_DN40097_c0	_g1_i2.p1.faa				
$258909\_HG4\_$ <b>G</b> ambierdisc	u <b>s</b> -	0	1	1	2
lapillus_DN22432_c0_g1_i2	.p1.faa				
$263811\_HG4\_$ <b>G</b> ambierdisc	u <b>s</b> -	1	0	1	2
lapillus_DN25138_c0_g1_i1	.p1.faa				
$319034\_HG4\_$ <b>G</b> ambierdisc	u <b>s</b> -	1	0	1	2
lapillus_DN40675_c3_g1_i2	.p1.faa				
$319505\_HG4\_$ <b>G</b> ambierdisc		1	0	1	2
lapillus_DN40711_c1_g8_i1	.p1.faa				
1041_MMETSIP0766_Gam	b <b>t</b> erdiscus-	0	0	1	2
$australes_DN11947_c0_g2_s$	i1.p1.faa				
27066_MMETSP0766_Ga	n $oldsymbol{artheta}$ ierdiscus-	0	0	1	2
australes_DN36729_c0_g1_	i1.p2.faa				
$274389\_HG4\_G$ ambierdisc	us-	2	0	0	2
lapillus_DN30113_c0_g1_i2	.p1.faa				

46553_MMETSP0766_Gambierdiscus-	0	0	0	2
australes_DN42196_c9_g4_i1.p1.faa				
148669_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN15462_c1_g7_i1.p1.faa				
234513_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN23482_c0_g1_i1.p1.faa				
63664_UTSMBR9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN10182_c0_g1_i1.p1.faa				
72166_UTSMBR9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN1258_c0_g1_i1.p1.faa				
210660_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18011_c6_g4_i1.p1.faa				
88291_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN13188_c2_g8_i2.p2.faa				
235070_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN25711_c0_g1_i1.p2.faa				
236919_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN33286_c0_g1_i1.p1.faa				
234708_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN24051_c0_g1_i1.p1.faa				
75892_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN12749_c1_g2_i3.p1.faa				
207498_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN17871_c4_g9_i11.p1.faa				
234298_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN22896_c0_g1_i1.p1.faa				
84448_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN13053_c3_g3_i4.p1.faa				
104611_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN13776_c4_g7_i11.p1.faa				

242597_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN9176_c0_g2_i2.p2.faa				
233698_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN2009_c0_g1_i1.p1.faa				
115505_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN14189_c2_g12_i1.p1.faa				
238946_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN4887_c0_g1_i1.p1.faa				
208524_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN17914_c1_g3_i4.p1.faa				
131131_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN14782_c2_g4_i1.p1.faa				
215621_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18221_c2_g6_i3.p1.faa				
225926_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18701_c1_g3_i2.p1.faa				
239297_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN5390_c0_g1_i1.p1.faa				
233616_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN19857_c0_g1_i1.p1.faa				
208525_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN17914_c1_g3_i5.p2.faa				
236171_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN30145_c0_g1_i1.p1.faa				
241217_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN7872_c0_g1_i1.p1.faa				
212813_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18098_c3_g3_i2.p1.faa				
147705_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN15422_c1_g3_i1.p1.faa				

242594_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN9176_c0_g1_i2.p1.faa				
86631_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN13131_c1_g1_i1.p1.faa				
238247_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN38343_c0_g1_i1.p1.faa				
212812_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18098_c3_g3_i1.p1.faa				
211703_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN18052_c3_g5_i1.p1.faa				
239230_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN5288_c0_g1_i1.p1.faa				
103957_UTSMER9A3_Gambierdiscus-	0	0	0	1
carpenteri_DN13754_c3_g2_i4.p1.faa				
462243_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN37930_c0_g1_i2.p1.faa				
$355979\_CG150Gambierdisc0s$ -	0	1	0	1
polynesiensis_DN10471_c0_g1_i1.p1.faa				
524904_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN43540_c1_g1_i2.p1.faa				
471036_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN38733_c0_g1_i1.p1.faa				
527904_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN43803_c0_g1_i1.p1.faa				
494332_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN40908_c1_g1_i1.p1.faa				
475327_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN39159_c1_g1_i1.p1.faa				
446377_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN36357_c3_g7_i1.p1.faa				

415511_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN33112_c0_g1_i3.p1.faa				
524930_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN43543_c1_g1_i4.p1.faa				
500254_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN41444_c1_g3_i1.p1.faa				
408903_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN32288_c3_g1_i1.p1.faa				
211708_UTSMER9A3_Gambierdiscus-	0	1	0	1
carpenteri_DN18052_c3_g5_i7.p1.faa				
524905_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN43540_c1_g1_i3.p1.faa				
528784_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN47453_c0_g1_i1.p3.faa				
528223_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN44935_c0_g1_i1.p2.faa				
362866_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN18821_c0_g1_i1.p1.faa				
408898_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN32288_c1_g1_i1.p1.faa				
473656_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN39000_c2_g2_i1.p1.faa				
505619_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN41913_c1_g3_i1.p2.faa				
357110_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN13123_c0_g1_i2.p2.faa				
529123_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN48937_c0_g1_i1.p1.faa				
419597_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN33575_c2_g1_i1.p1.faa				

486622_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN40207_c2_g2_i2.p1.	faa				
518712_CG150Gambierdisctos-	0	1	0	1	
polynesiensis_DN43045_c0_g2_i6.p1.	faa				
505617_CG150Gambierdiscos-	0	1	0	1	
polynesiensis_DN41913_c1_g2_i1.p1.	faa				
419857_CG150Gambierdisc <b>0</b> s-	0	1	0	1	
polynesiensis_DN33604_c1_g1_i1.p1.	faa				
319033_HG4_ <b>G</b> ambierdiscu <b>©</b>	0	1	0	1	
lapillus_DN40675_c3_g1_i1.p1.faa					
505612_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN41913_c0_g1_i1.p1.	faa				
505621_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN41913_c1_g5_i1.p2.	faa				
368243_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN21805_c0_g1_i1.p1.	faa				
531066_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN7198_c0_g1_i1.p1.fa	aa				
411779_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN32643_c5_g2_i3.p2.	faa				
529709_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN51840_c0_g1_i1.p1.	faa				
424815_CG150Gambierdisct0s-	0	1	0	1	
polynesiensis_DN34144_c0_g1_i6.p1.	faa				
$388829$ _CG1 $50$ Gambierdisc $0$ s-	0	1	0	1	
polynesiensis_DN29147_c0_g1_i1.p1.	faa				
$528991$ _CG1 $50$ Gambierdisc $0$ s-	0	1	0	1	
polynesiensis_DN4849_c0_g1_i1.p2.fa	aa				
529886_CG150Gambierdiscus-	0	1	0	1	
polynesiensis_DN52795_c0_g1_i1.p1.	faa				

517572_CG150Gambierdiscos-	0	1	0	1
polynesiensis_DN42942_c0_g1_i1.p1.faa				
162319_UTSMER9A3_Gambierdiscus-	0	1	0	1
carpenteri_DN15967_c2_g1_i1.p1.faa				
486374_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN40177_c0_g2_i3.p1.faa				
424977_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN34166_c0_g6_i1.p2.faa				
480000_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN39607_c0_g2_i4.p1.faa				
524933_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN43543_c1_g1_i7.p1.faa				
529340_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN50363_c0_g1_i1.p1.faa				
382787_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN27509_c0_g1_i1.p1.faa				
455767_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN37290_c0_g4_i1.p1.faa				
454667_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN37192_c1_g3_i1.p1.faa				
505616_CG150Gambierdisct0s-	0	1	0	1
polynesiensis_DN41913_c1_g1_i3.p1.faa				
408904_CG150Gambierdisct0s-	0	1	0	1
polynesiensis_DN32288_c3_g2_i1.p1.faa				
519735_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN43127_c3_g5_i1.p1.faa				
524932_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN43543_c1_g1_i6.p1.faa				
419608_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN33575_c2_g2_i1.p1.faa				

489214_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN40447_c0_g1_i2.p1.faa				
407098_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN32057_c0_g1_i2.p1.faa				
486620_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN40207_c2_g1_i2.p2.faa				
529847_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN52688_c0_g1_i1.p1.faa				
355910_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN1036_c0_g1_i1.p2.faa				
419599_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN33575_c2_g1_i11.p1.faa				
368244_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN21805_c0_g2_i1.p1.faa				
528301_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN45312_c0_g1_i1.p1.faa				
431157_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN34812_c2_g1_i1.p1.faa				
429838_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN3467_c0_g1_i1.p1.faa				
485799_CG150Gambierdisc <b>0</b> s-	0	1	0	1
polynesiensis_DN40132_c0_g3_i1.p1.faa				
449384_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN36673_c0_g1_i3.p1.faa				
530384_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN55090_c0_g1_i1.p1.faa				
357109_CG150Gambierdisc@s-	0	1	0	1
polynesiensis_DN13123_c0_g1_i1.p2.faa				
466543_CG150Gambierdisc0s-	0	1	0	1
polynesiensis_DN38313_c1_g3_i1.p1.faa				

367731_CG150Gambierdiscos-	0	1	0	1
polynesiensis_DN21547_c0_g1_i1.p1.faa				
438506_CG150Gambierdiscos-	0	1	0	1
polynesiensis_DN35575_c0_g1_i7.p1.faa				
491823_CG150Gambierdiscus-	0	1	0	1
polynesiensis_DN40690_c4_g5_i2.p1.faa				
530249_CG150Gambierdiscos-	0	1	0	1
polynesiensis_DN54681_c0_g1_i1.p1.faa				
661643_HG5_Cambierdiscu	0	0	1	1
silvae_DN57114_c0_g1_i1.p1.faa				
601478_HG5_Cambierdiscu	0	0	1	1
silvae_DN43780_c7_g8_i1.p1.faa				
$567939\_HG5\_G$ ambierdiscu $\Theta$	0	0	1	1
silvae_DN35530_c0_g3_i1.p1.faa				
593688_HG5_Cambierdiscus	0	0	1	1
silvae_DN42661_c0_g1_i1.p1.faa				
540524_HG5_Gambierdiscus	0	0	1	1
silvae_DN20879_c0_g2_i1.p1.faa				
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silvae_DN48408_c0_g1_i4.p1.faa				
620146_HG5_Gambierdiscus	0	0	1	1
silvae_DN45801_c1_g1_i1.p2.faa				
589550_HG5_Cambierdiscus	0	0	1	1
silvae_DN41996_c3_g12_i1.p1.faa				
643868_HG5_Gambierdiscus	0	0	1	1
silvae_DN47931_c1_g3_i5.p1.faa				
657026_HG5_Cambierdiscus	0	0	1	1
silvae_DN48988_c0_g3_i1.p1.faa				
589562_HG5_Cambierdiscus	0	0	1	1
$silvae\_DN41996\_c3\_g5\_i1.p2.faa$				

608846_HG5_Cambierdiscu	0	0	1	1
silvae_DN44648_c2_g1_i1.p1.faa				
593690_HG5_Cambierdiscu	0	0	1	1
silvae_DN42661_c0_g2_i3.p1.faa				
550256_HG5_Gambierdiscus	0	0	1	1
silvae_DN27602_c0_g2_i1.p1.faa				
608853_HG5_Cambierdiscu	0	0	1	1
silvae_DN44648_c2_g6_i1.p1.faa				
559711_HG5_@ambierdiscu@	0	0	1	1
silvae_DN32102_c0_g1_i2.p1.faa				
575231_HG5_@ambierdiscu@	0	0	1	1
silvae_DN38322_c1_g2_i1.p1.faa				
591087_HG5_@ambierdiscu@	0	0	1	1
silvae_DN42232_c1_g4_i1.p2.faa				
657027_HG5_Gambierdiscus	0	0	1	1
silvae_DN48988_c0_g3_i2.p1.faa				
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silvae_DN20879_c0_g3_i1.p1.faa				
601479_HG5_@ambierdiscu@	0	0	1	1
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lapillus_DN10661_c0_g1_i1.p1.faa				
354441_HG4_Cambierdiscu	1	0	0	1
lapillus_DN7536_c0_g1_i1.p2.faa				

277633_HG4_Cambierdiscu	1	0	0	1
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lapillus_DN40082_c0_g1_i1.p1.faa				
319501_HG4_Cambierdiscu	1	0	0	1
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lapillus_DN41459_c1_g5_i1.p1.faa				

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lapillus_DN25642_c0_g1_i1.p1.faa				
310329_HG4_Cambierdiscu	1	0	0	1
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298246_HG4_Cambierdiscus	1	0	0	1
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lapillus_DN40082_c0_g2_i1.p1.faa				
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35353_MMETSP0766_Gambierdiscus-	0	0	0	1
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15763_MME	TSP0766_Gar	n <b>ð</b> ierdiscus-	0	0	0	1
australes_DN	$127921_{c}0_{g}1_{c}$	i1.p1.faa				
41417_MME	TSP0766_Gar	n $oldsymbol{artheta}$ ierdiscus-	0	0	0	1
australes_DN	V41862_c2_g2_	i1.p1.faa				
28600_MME	TSP0766_Gar	n <b>ð</b> ierdiscus-	0	0	0	1
australes_DN	$137530_{c}0_{g}2_{c}$	i1.p1.faa				
7400_MMET	SIP0766_Gam	b@erdiscus-	0	0	0	1
australes_DN	V21004_c0_g1_	i1.p1.faa				
35356_MME	TSP0766_Gar	n <b>∂</b> ierdiscus-	0	0	0	1
australes_DN	$140756_{-}c0_{-}g4_{-}$	i1.p1.faa				
30416_MME	TSP0766_Gar	n <b>ð</b> ierdiscus-	0	0	0	1
australes_DN	V38640_c0_g3_	i1.p1.faa				
18686_MME	TSP0766_Gar	n $oldsymbol{artheta}$ ierdiscus-	0	0	0	1
australes_DN	V30415_c0_g2_	i1.p1.faa				
16842_MME	TSP0766_Gar	n $oldsymbol{artheta}$ ierdiscus-	0	0	0	1
australes_DN	\\\28731_c0_g3_	i1.p2.faa				

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