Chapter 5: Using transcriptomics to investigate evolution and toxicology in *Gambierdiscus*. [1]

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 holmesii* 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 *Prorocentrum 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. carpenteri*	*G. lapillus*	*G. polynesiensis*	*G. holmesii*
Strain	CAWD149	UTSMER9A	HG4	CG15	HG5
Transcriptome source	MMETSP	**chapter 4**	**chapter 4**	**chapter 4**	**chapter 4**
Accession ID	MMETSP0766	SRR6821720	SRR6821722	SRR6821723	SRR6821721
Isolation location	Rarotonga, Cook Islands (2007)	Merimbula, Australia (2014)	Heron Island, Australia (2014)	Rarotonga, Cook Islands (2014)	Heron Island, Australia (2014)
Toxin profile (LC-MS/MS)	CTX -ve; MTX +ve	CTX -ve; MTX -ve	CTX -ve; MTX +ve	CTX +ve; MTX +ve	CTX -ve; MTX +ve
Toxicity via bioassay	CTX +ve; MTX N/A	CTX -ve; MTX +ve	CTX +ve*; MTX +ve*	CTX +ve*; MTX +ve*	CTX +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).

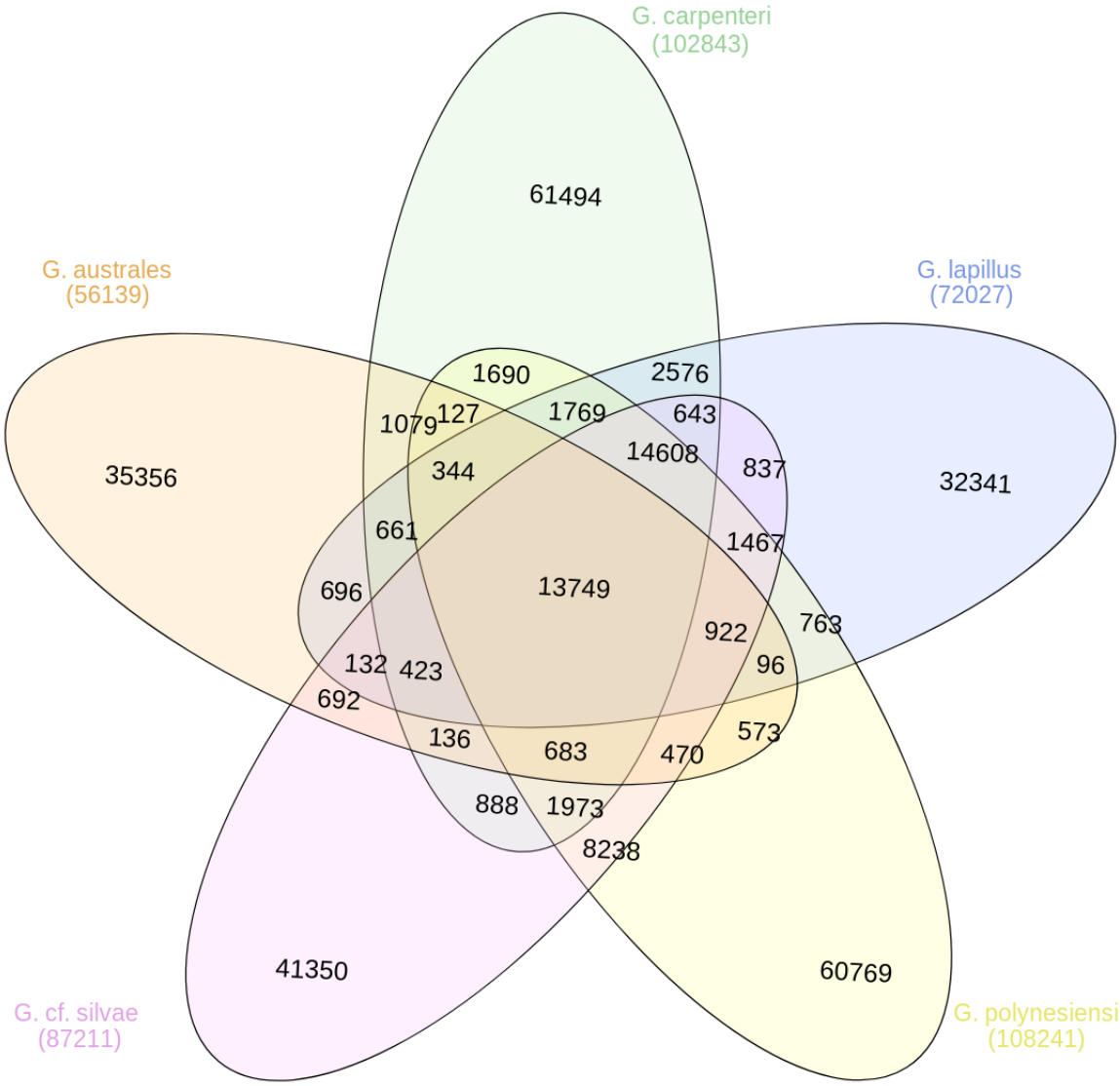

Figure 1: Venn diagram of species distribution across clusters.

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

Species	*G. australes*	*G. carpenteri*	*G. lapillus*	*G. polynesiensis*	*G. holmesii*
Contigs	102,863	263,829	148,972	270,315	191,224
Spliced leader contigs	304	683	232	1,570	1,524
Nucleotide clusters (cd-hit)	102,861	263,743	148,966	270,265	191,205
Predicted coding regions (Transdecoder)	63,299	180,568	111,862	176,290	132,688
Contigs annotated (Interpro Scan)	131,970	334,737	225,324	225,324	254,844
Core-transcriptome clusters	13,750	13,750	13,750	13,750	13,750
Softcore-transcriptome clusters	2,372	16,058	16,297	16,557	16,636
Unique clusters	35,356	61,494	32,341	60,769	41,350

Tim: It seems kinda conspicuous that the unique clusters of *G. carpenteri* & *G. poly* are almost twice the number of *G. lapillus* and *G. holmesii*, 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. holmesii* 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. holmesii* 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. holmesii* 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*.

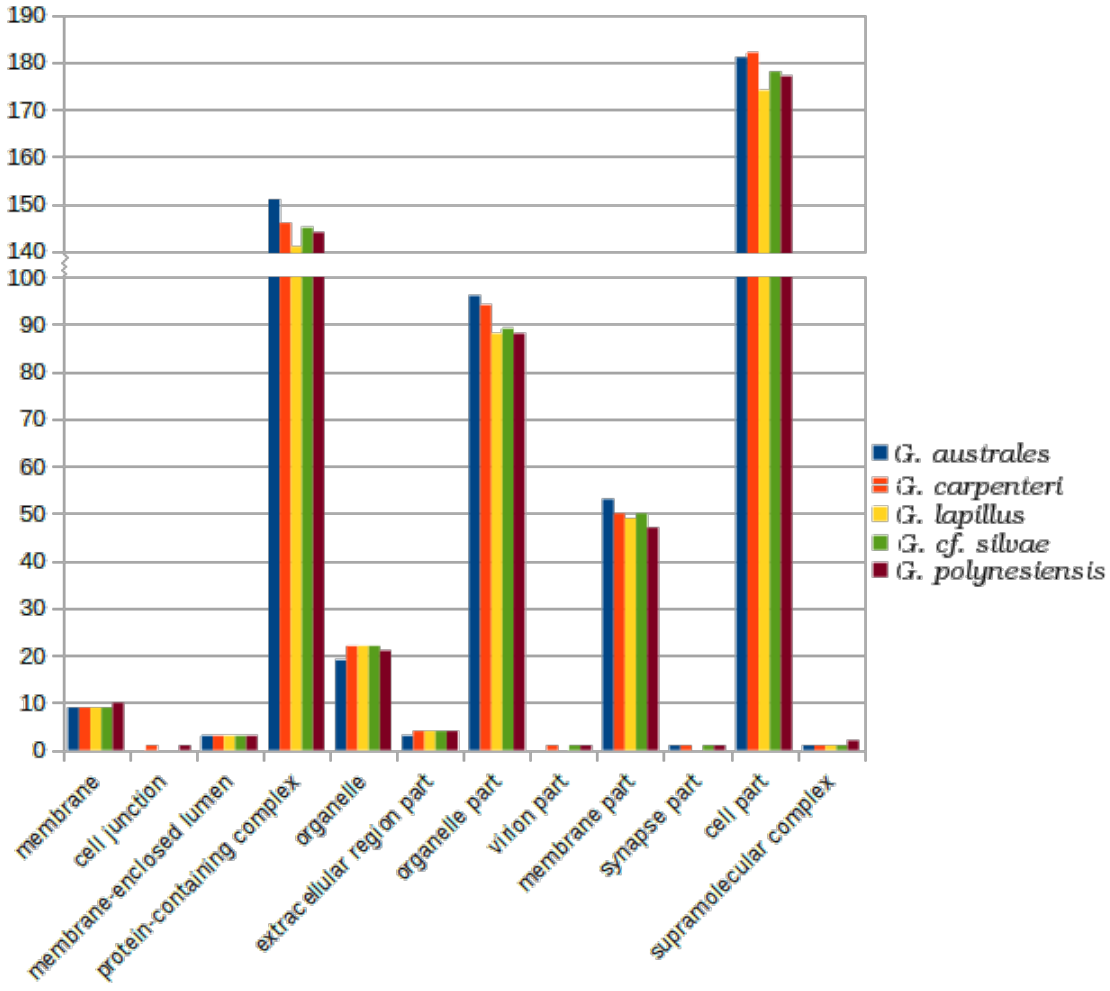

Figure 2: Summary of cellular GO annotations between *Gambierdiscus* species at GO-SUM level 1 from Suppl. table 3.

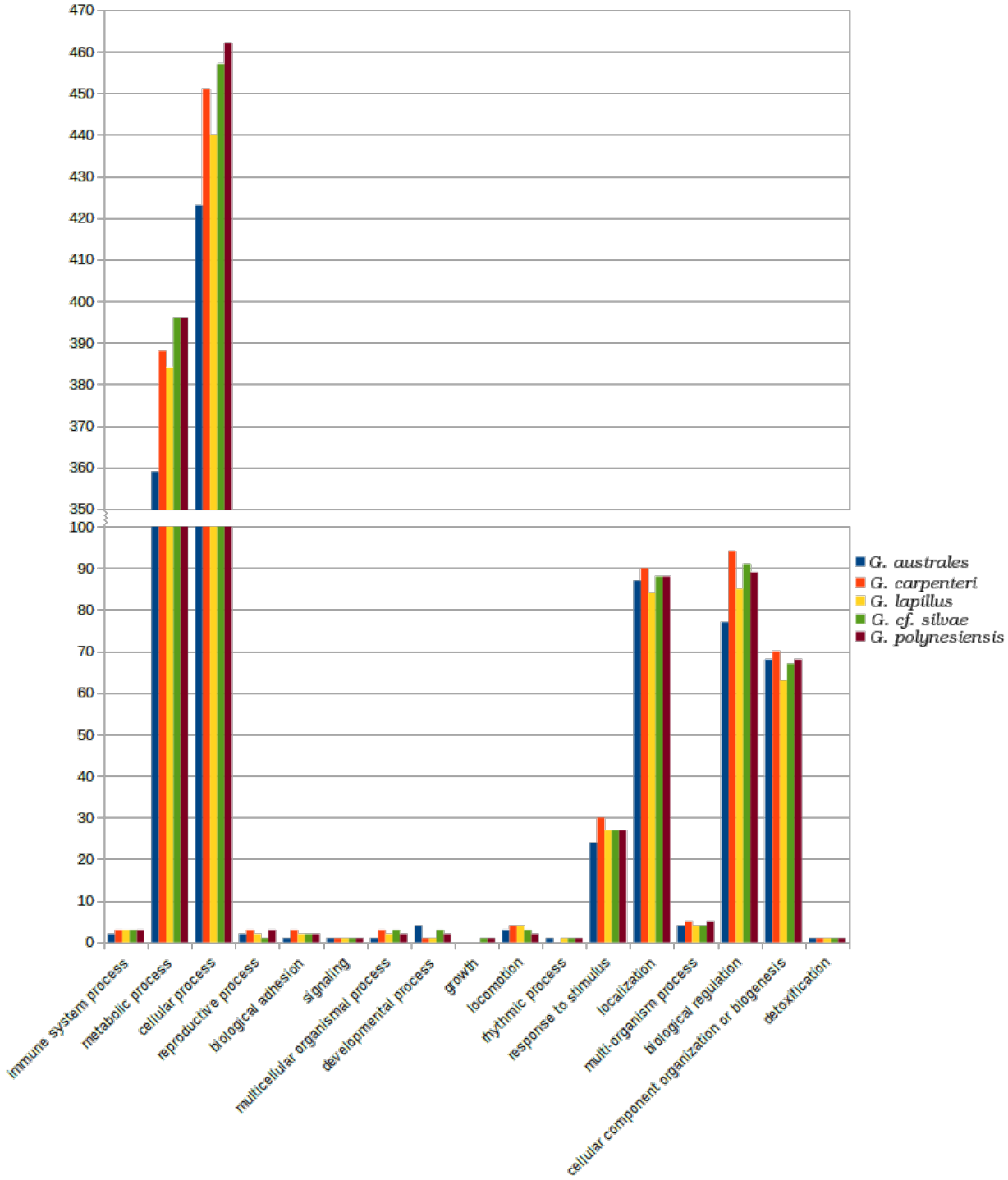

Figure 3: Summary of biological processes GO annotations between *Gambierdiscus* species at GOSUM level 1 from Suppl. table 3.

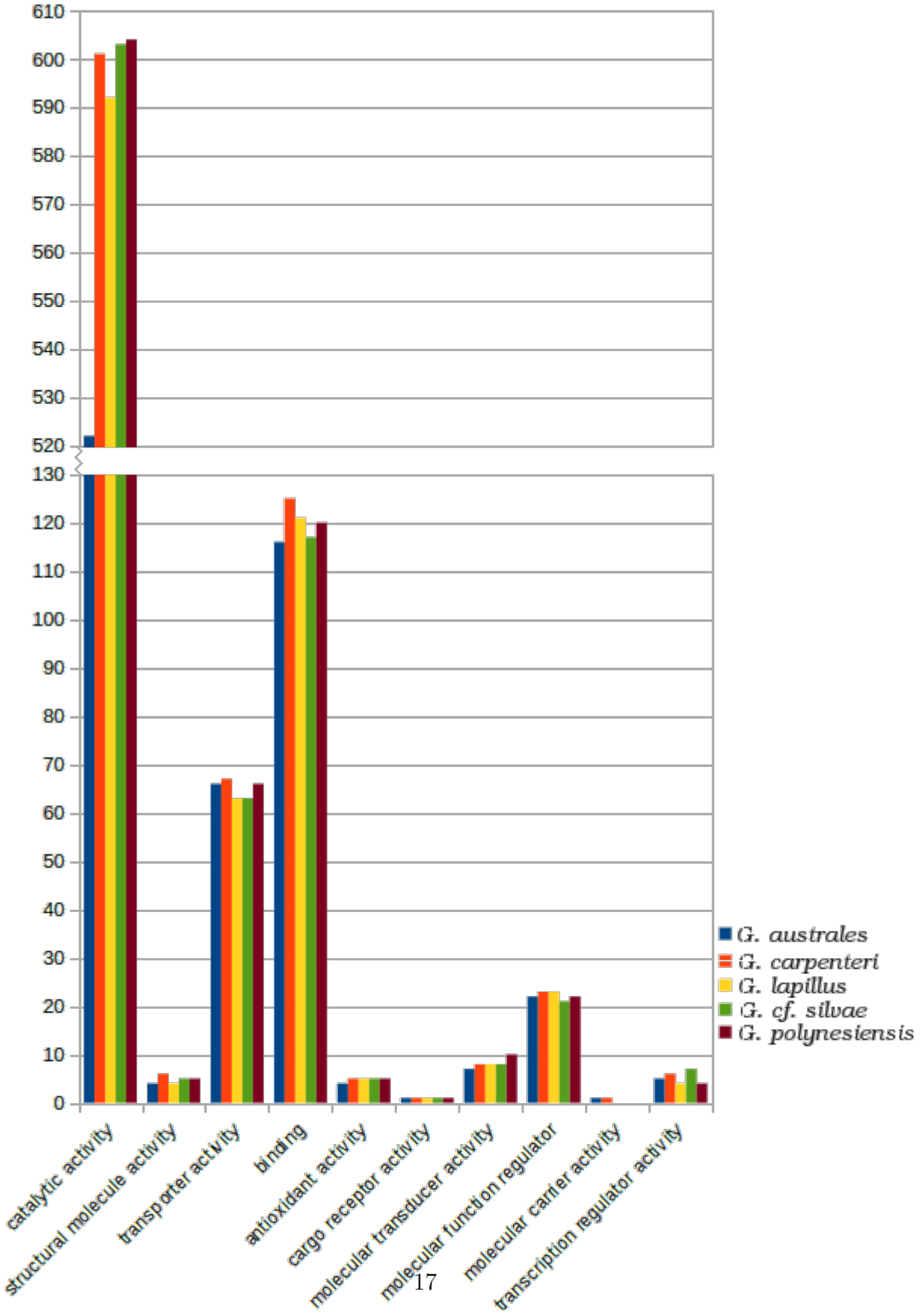

Figure 4: Summary of molecular GO annotations between *Gambierdiscus* species at GOSUM level 1 from Suppl. table 3.

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 inttracellular 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).

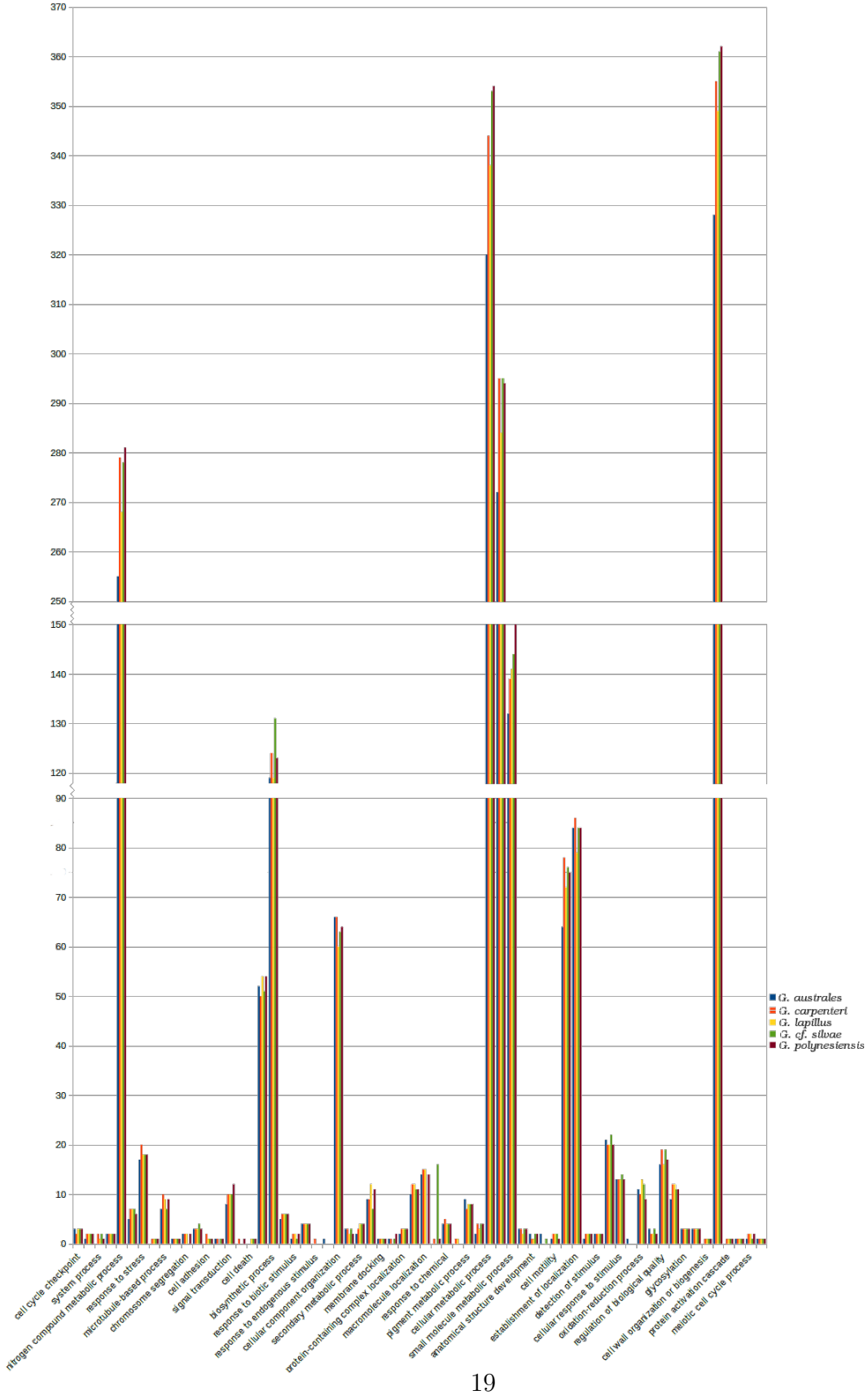

Figure 5: Summary of biological processes GO annotations between *Gambierdiscus* species at GOSUM level 2 from Suppl. table 4.

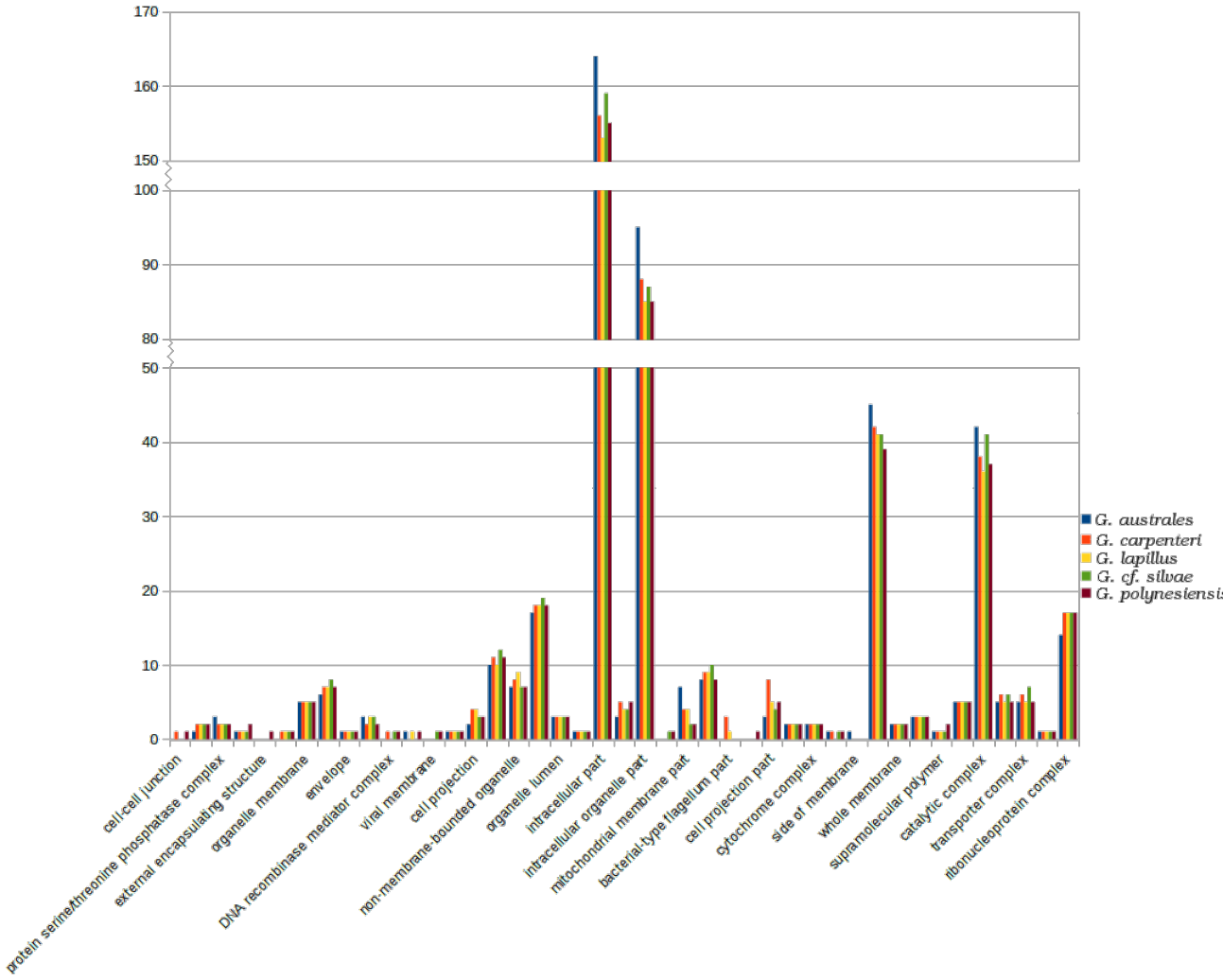

Figure 6: Summary of cellular GO annotations between *Gambierdiscus* species at GO-SUM level 2 from Suppl. table 4.

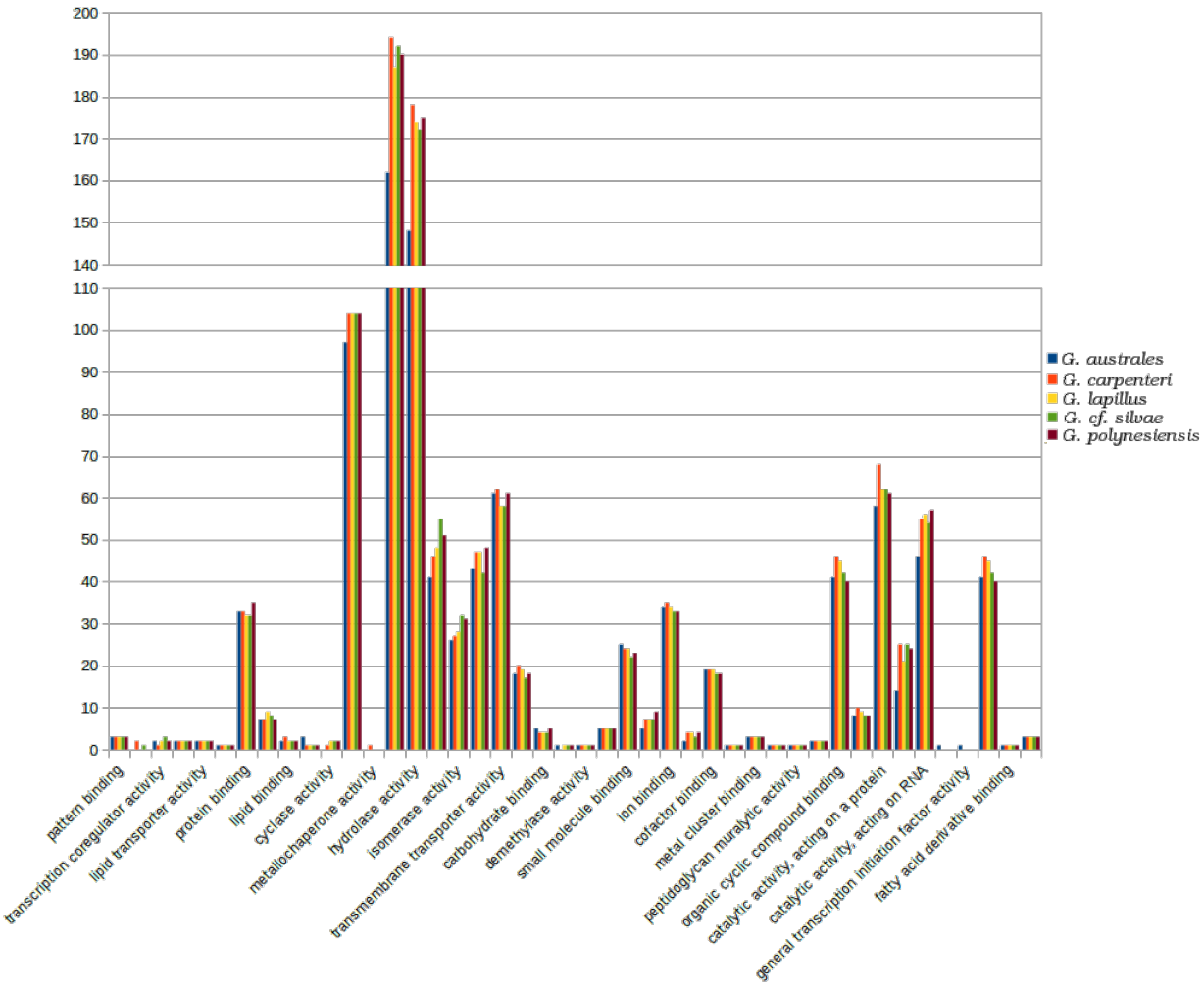

Figure 7: Summary of molecular GO annotations between *Gambierdiscus* species at GOSUM level 2 from Suppl. table 4.

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).

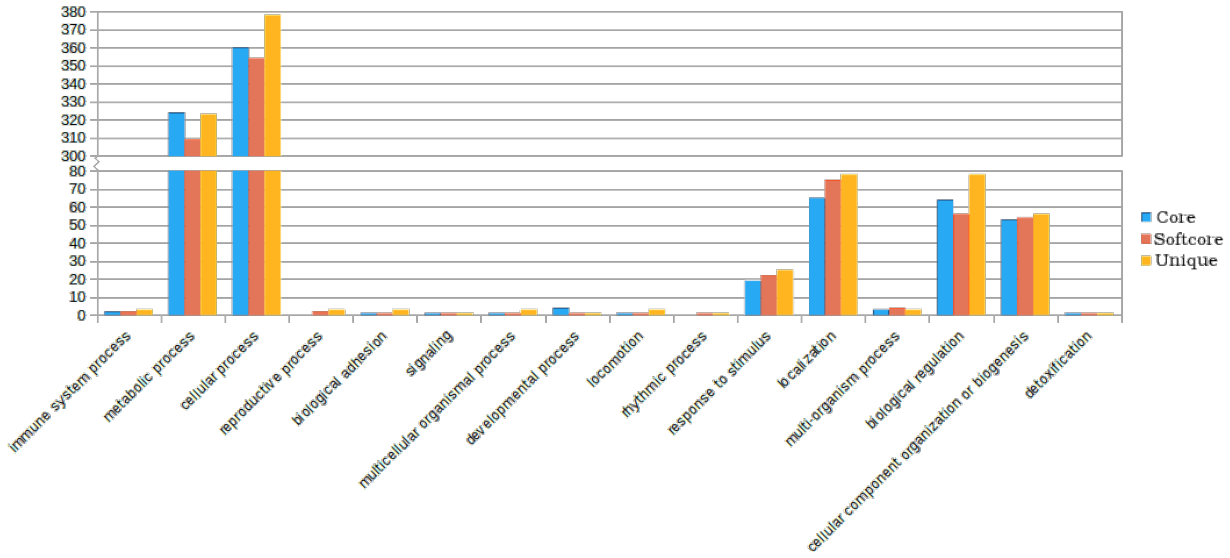

Figure 8: Summary of biological processes GO annotations between core, softcore and unique clusters at GOSUM level 1.

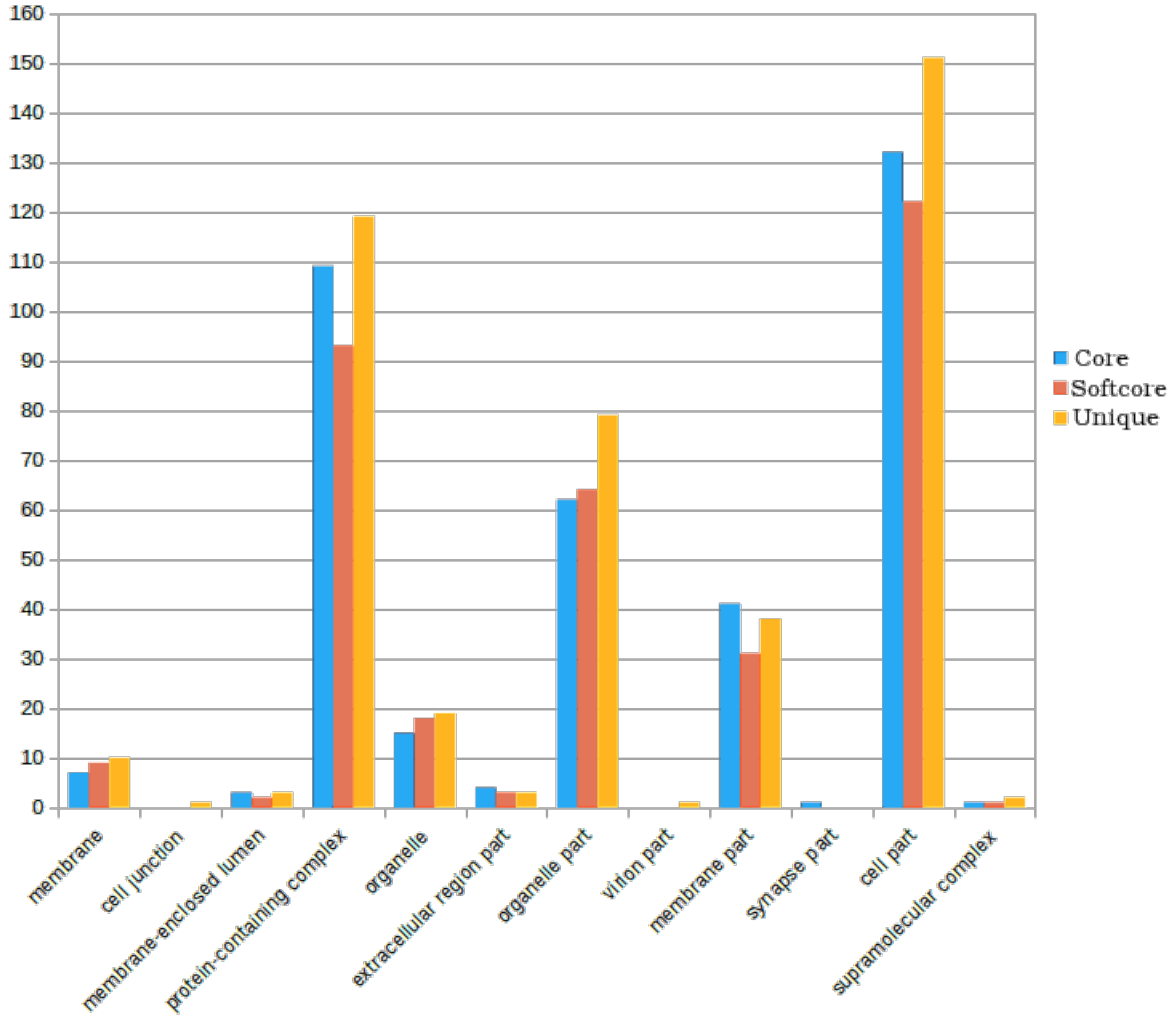

Figure 9: Summary of cellular GO annotations between core, softcore and unique clusters at GOSUM level 1.

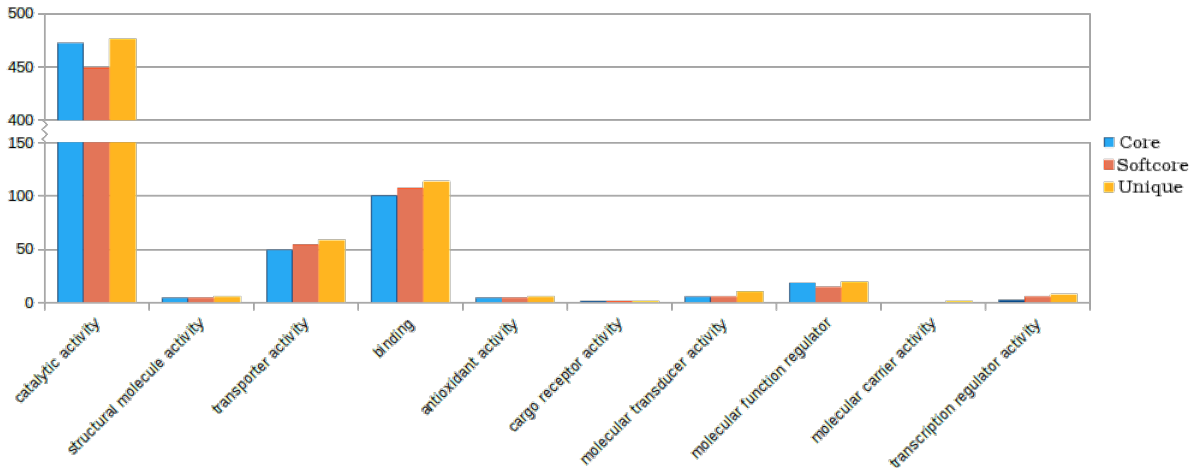

Figure 10: Summary of molecular GO annotations between core, softcore and unique clusters at GOSUM level 1.

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 motilityand a protein activation cascade.

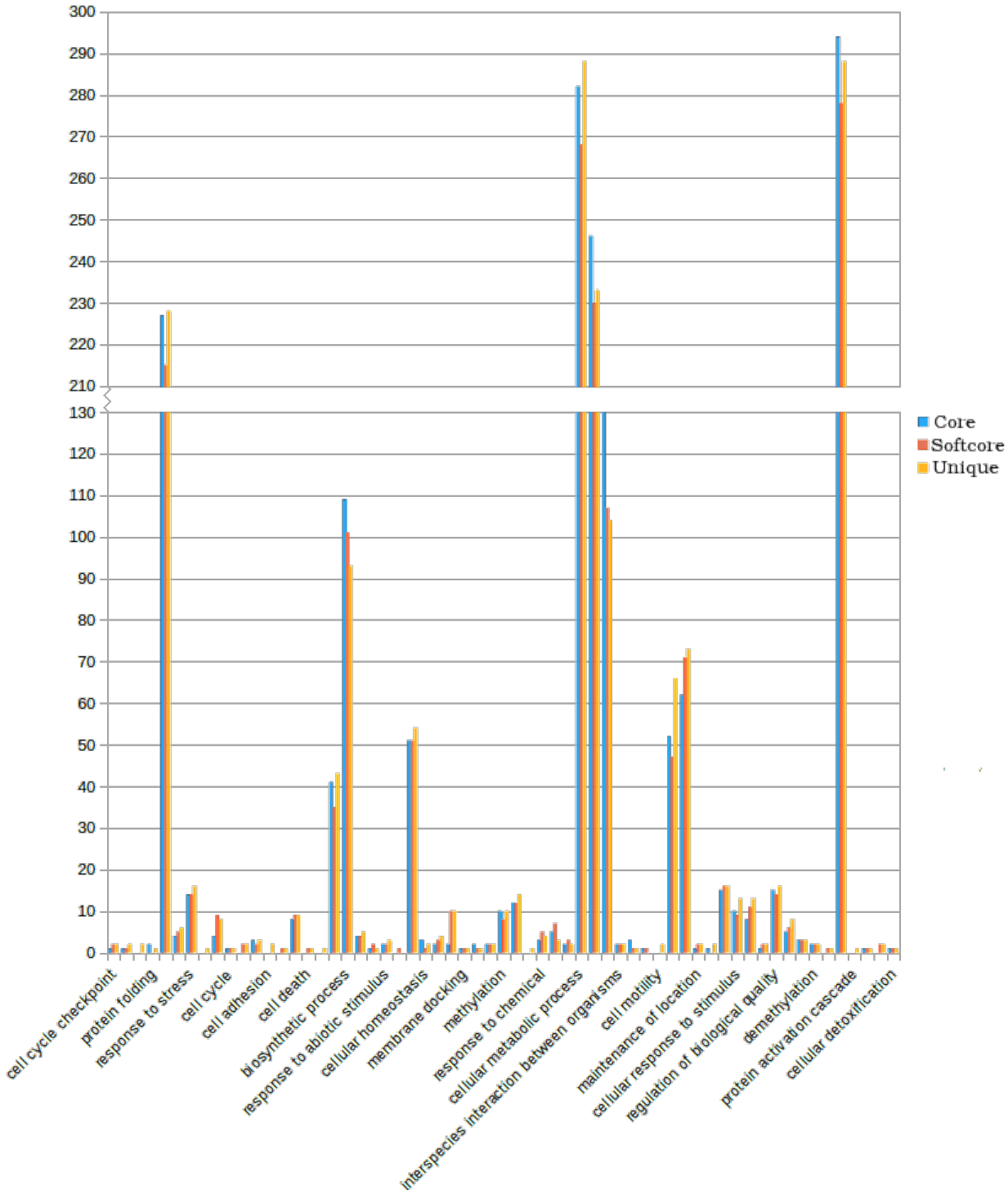

Figure 11: Summary of biological processes GO annotations between core, softcore and unique clusters at GOSUM level 2.

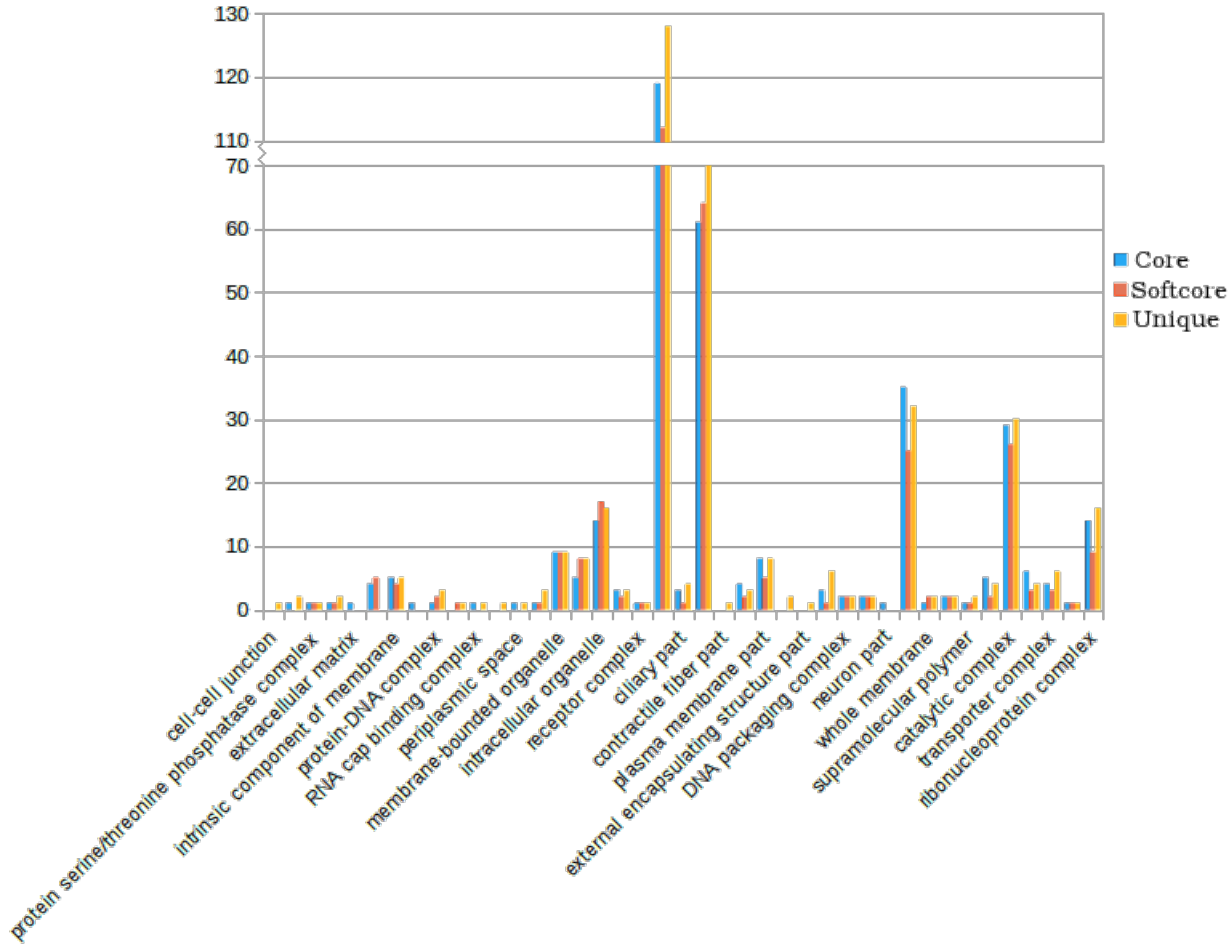

Figure 12: Summary of cellular GO annotations between core, softcore and unique clusters at GOSUM level 2.

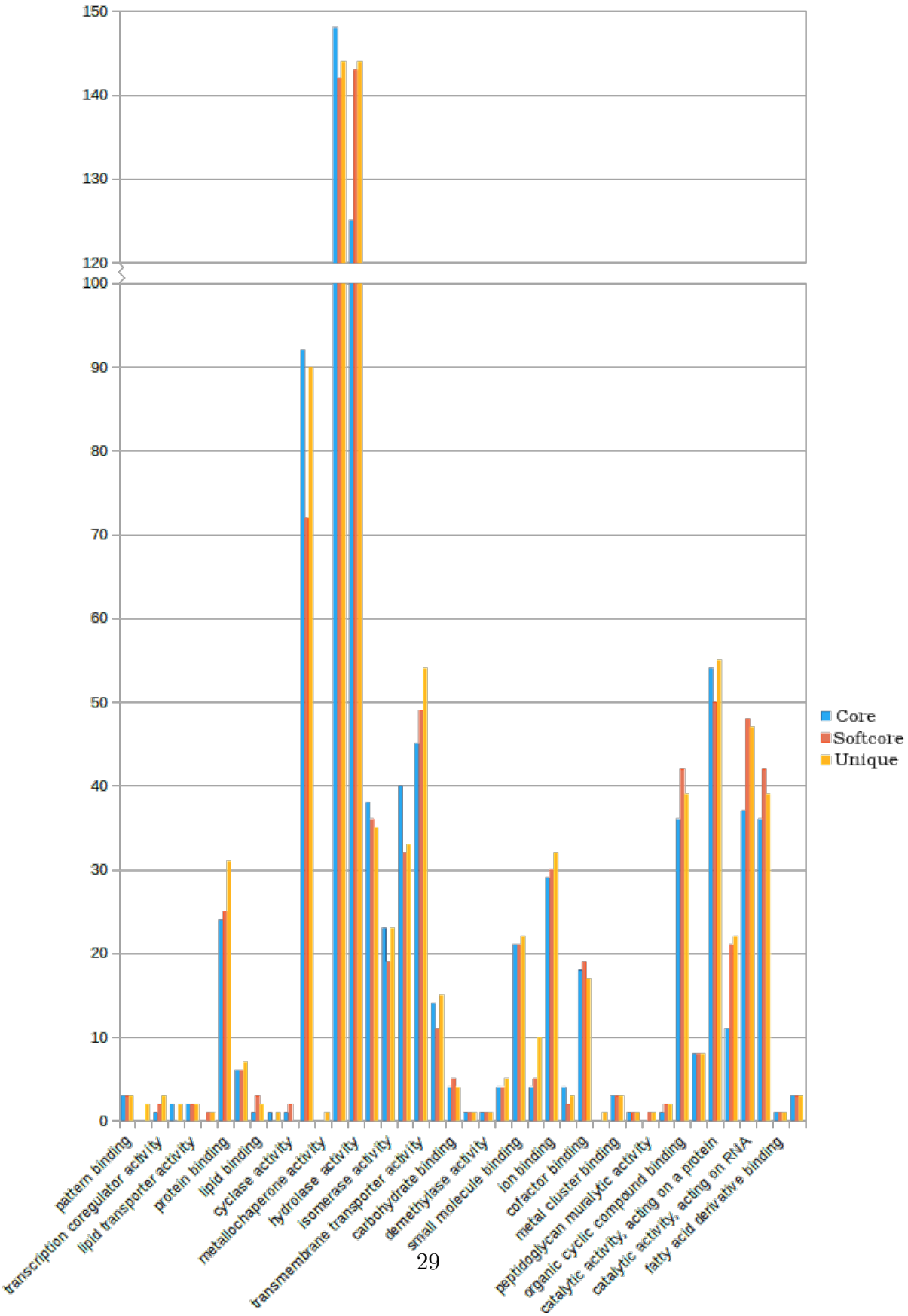

Figure 13: Summary of molecular GO annotations between core, softcore and unique clusters at GOSUM level 2.

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. holmesii* with equally represented transcript numbers. Four contigs contained *G. polynesiensis* and *G. holmesii* only, one of which had a higher contig representation of *G. polynesiensis* than *G. holmesii*. *G. polynesiensis* was the only representative species in 71 clusters, of which three clusters contained 2 contigs and one cluster contained 3 contigs. *G. holmesii* 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.

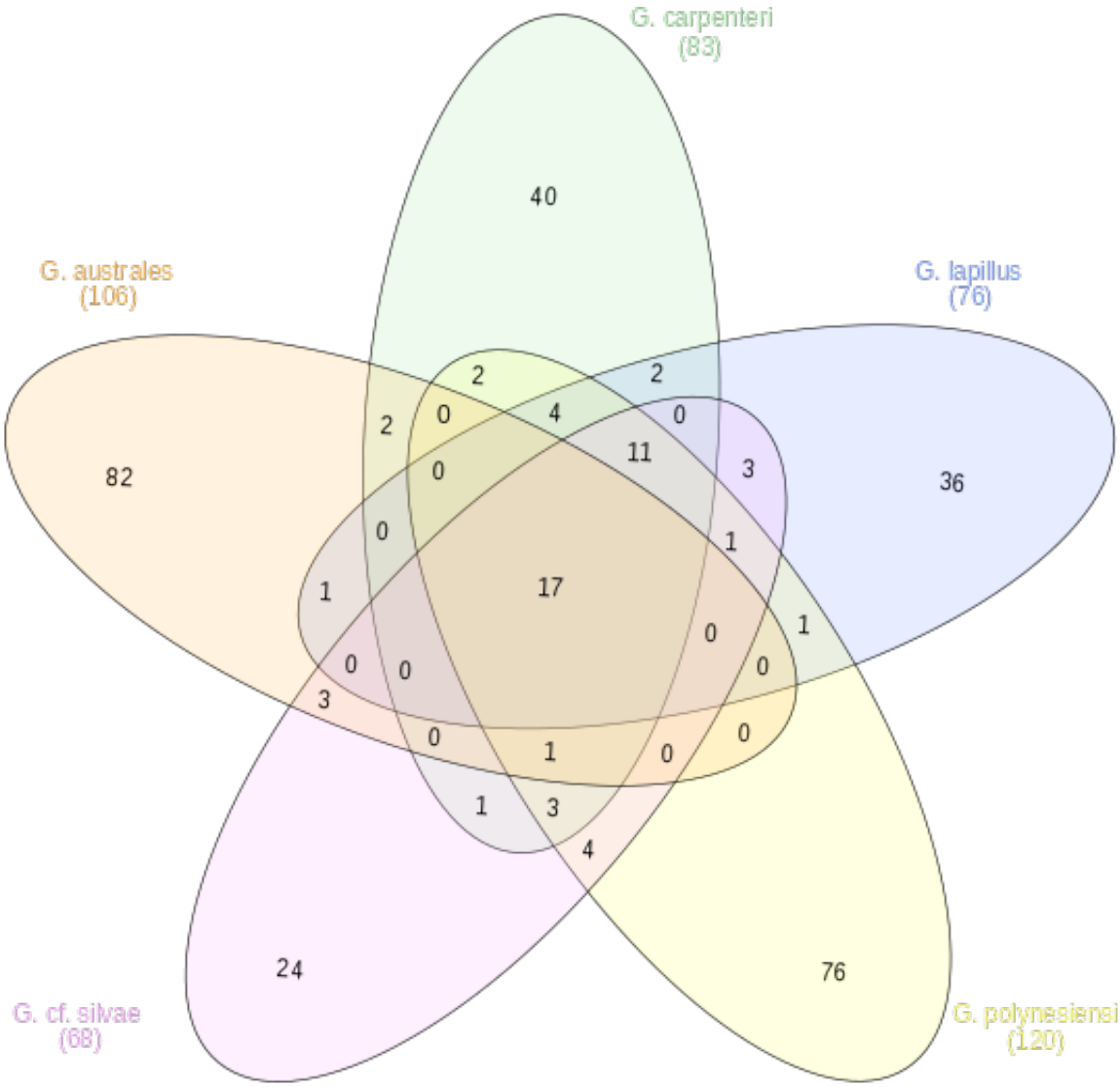

Figure 14: Venn diagram of species in KS clusters.

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. The number of predicted peptides found in this study is high compared to a pan-transcriptome study of four prymnesiophyte algae [20]. The predicted peptides in the Koid et al. (2014) study ranged from around 25,000 to 56,000 peptides, where this study found a range from about 63,000 to 270,000 predicted peptides (table 2). The lowest number of peptides was predicted in *G. australes*, similar to the findings in the prymnesiophyte algae study and both originating from the MMTESP sequenced with 50bp read length. *G. holmesii* and *G. lapillus* predicted peptides numbered 132,688 and 111,862 respectively, sequenced with 75bp read length. The highest number of predicted peptides were found in *G. capenteri* and *G. polynesiensis* at 180,568 and 176,290 respectively, sequenced with 150bp read length. Hence the differences in predicted peptide numbers could be linked to sequencing depth based on the read lenths used, which is supported by the comparable predicted peptide recovery from *G. australes* and the prymnesiophyte algae.

The abundance of dinoSL was quite low (table 2) compared to the abundance observed by Zhang et al. (2009) in *Amphidinium carterae*. Similar to this study, low abundance of spliced leaders has been observed in other dinoflagellates [2, 9]. The function of the spliced leader, and hence whether this variation in observation between high and low abundance is species specific or due to assembly method employed, is yet unknown. Interestingly, *G. homesii* and *G. polynesiensis* had the most contigs with dinoSLs. These were sequenced with different read lengths and collected from different geographic locations, but are phylogenetically in the same sub-clade.

5.1 Core, soft-core and unique genes

The number of contigs and predicted peptides from this study was markedly less for *G. australes*, 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 *Gambierdiscus* pan-transcriptome core which was not captured in the *G. australes* sequencing. This is an example where the relevance of a reference pan-transcriptome for sequencing efforts becomes evident.

There was no distinct difference between the species GO annotations, with the exception of *G. australes* which is likely due to lower overall contig recovery from the MMETSP sequencing data which translated to shallower sequencing depth. This is as expected, as the species operate under similar nutritional modes and within similar temperature ranges, apart from *G. carpenteri* which was isolated from the temperate Merimbula, NSW, region rather than the tropical or sub-tropical conditions from which *G. australes*, *G. holmesii*, *G. carpenteri* and *G. polynesiensis* hail. However no observable difference was observed in the biological, cellular and molecular GO annotation groups at levels 1 and 2 between the core, soft-core and unique either. This is somewhat less expected as as it would be reasonable to predict a functional difference for transiently expressed genes unique to each species. Possible reasons could be that this observation only captures predicted peptides with GO annotations which were only 15.23 % of the 231,310 unique clusters. This indicates that no functional match for over 196,000 of the unique clusters could be found and no indication what their function might be could be extrapolated.

5.2 Ketosynthase domain detection.

5.3 Areas for possible improvement in this study.

This chapter represents a novel approach to analyzing *Gambierdiscus* transcriptomes and possible avenues for investigation for ciguatoxin production pathways. However there are several aspects that can be improved upon with future studies.

Contaminants in dataset. The RNA-seq libraries were constructed from whole RNA seq runs with non-axenic cultures, hence it is likely that bacterial RNA is a subset of the analysis. It is unlikely that the same contamination persists in the core and softcore clusters, i.e. across all four to five species collected from Australia, the Cook Islands and over a 10 year time span. However the unique clusters could well be contaminated with bacterial contigs. Hence it would be pertinent to utilize a eukaryote specific RNA-seq strategy for future studies, or devise a method to separate bacterial from eukaryotic

contigs post sequencing.

Coverage of taxa. As with bacterial pan-transcriptomics, eukaryotic studies reveal a correlation between the number of species and strains included for refining the core transcriptome and expanding the unique fraction of the transcriptome []. The importance of including several strains of a species becomes apparent with the discovery of a non-ciguatoxic *G. polynesiensis* strain []. Variability in morphology and toxicity has also been observed in *G. lapillus* [21]. While this study sought to cover taxa from the three main *Gambierdiscus* clades, an increase in species and strain coverage is highly likely to impact the resolution of the core and unique portions especially as the sequencing coverage of the *G. australes* transcriptome is less in depth than the other four species (table 2). Hence due to limited species and the singular strain per species coverage, this represents an exploratory study for establishing a pan-transcriptome for *Gambierdiscus* which should be improved upon.

PKS active domain search. PKS complexes consist of a number of active domains that synthesize and manipulate the polyketide backbone. The KS domain is but one of several essential domains for a functional polyketide. The search for active domains should be extended to include other domains for comparison in the search for the ciguatoxin production pathways.

Conclusion

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

Supplementary

- need to add australes

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

GO accession	GO terms	*G. carpenteri*	*G. lapillus*	*G. polynesiensis*	*G. holmesii*
Biological processes					
GO:0002376	immune system process	3	3	3	3
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 organismal process	3	2	3	2
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 process	5	4	4	5
GO:0065007	biological regulation	94	85	91	89
GO:0071840	cellular component organization/biogenesis	70	63	67	68
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 lumen	3	3	3	3
GO:0032991	protein-containing complex	146	141	145	144

GO:0043226	organelle	22	22	22	21
GO:0044421	extracellular region part	4	4	4	4
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 complex	1	1	1	2
Molecular function					
GO:0003824	catalytic activity	601	592	603	604
GO:0005198	structural molecule activity	6	4	5	5
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 activity	8	8	8	10
GO:0098772	molecular function regulator	23	23	21	22
GO:0140104	molecular carrier activity	1	0	0	0
GO:0140110	transcription regulator activity	6	4	7	4

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

GO accession	GO terms	*G. carpenteri*	*G. lapillus*	*G. polynesiensis*	*G. holmesii*
Biological processes					
GO:0000075	cell cycle checkpoint	2	3	3	3
GO:0002252	immune effector process	2	2	2	2
GO:0003008	system process	2	1	2	1
GO:0006457	protein folding	2	2	2	2
GO:0006807	nitrogen compound metabolic process	279	268	278	281
GO:0006928	movement of cell or subcellular component	7	7	7	6
GO:0006950	response to stress	20	18	18	18
GO:0006955	immune response	1	1	1	1
GO:0007017	microtubule-based process	10	9	7	9
GO:0007049	cell cycle	1	1	1	1
GO:0007059	chromosome segregation	2	2	0	2
GO:0007154	cell communication	3	3	4	3
GO:0007155	cell adhesion	2	1	1	1
GO:0007163	establishment or maintenance of cell polarity	1	0	1	1
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 stimulus	6	6	6	6
GO:0009607	response to biotic stimulus	2	2	1	2
GO:0009628	response to abiotic stimulus	4	4	4	4
GO:0009719	response to endogenous stimulus	1	0	0	0
GO:0016043	cellular component organization	66	60	63	64
GO:0019725	cellular homeostasis	3	2	3	2
GO:0019748	secondary metabolic process	3	4	4	4
GO:0022402	cell cycle process	9	12	7	11
GO:0022406	membrane docking	1	1	1	1
GO:0030029	actin filament-based process	1	0	1	2
GO:0031503	protein-containing complex localization	3	3	3	3
GO:0032259	methylation	12	12	11	11
GO:0033036	macromolecule localization	15	15	0	14
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 process	7	8	8	8
GO:0044085	cellular component biogenesis	4	3	4	4
GO:0044237	cellular metabolic process	344	338	353	354

GO:0044238	primary metabolic process	295	284	295	294
GO:0044281	small molecule metabolic process	139	141	144	150
GO:0044419	interspecies interaction between organisms	3	2	3	3
GO:0048856	anatomical structure development	1	1	2	2
GO:0048869	cellular developmental process	0	0	1	
GO:0048870	cell motility	2	2	2	1
GO:0050789	regulation of biological process	78	72	76	75
GO:0051234	establishment of localization	86	79	84	84
GO:0051235	maintenance of location	2	2	2	2
GO:0051606	detection of stimulus	2	2	2	2
GO:0051641	cellular localization	20	20	22	20
GO:0051716	cellular response to stimulus	13	13	14	13
GO:0055114	oxidation-reduction process	10	13	12	9
GO:0061919	process utilizing autophagic mechanism	2	2	3	2
GO:0065008	regulation of biological quality	19	16	19	17
GO:0065009	regulation of molecular function	12	12	11	11
GO:0070085	glycosylation	3	3	3	3

GO:0070988	demethylation	3	3	3	3
GO:0071554	cell wall organization or biogenesis	1	1	1	1
GO:0071704	organic substance metabolic process	355	349	361	362
GO:0072376	protein activation cascade	1	1	1	1
GO:0140029	exocytic process	1	1	1	1
GO:1903046	meiotic cell cycle process	2	2	1	2
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 serine/threonine phosphatase complex	2	2	2	2
GO:0019867	outer membrane	1	1	1	2
GO:0030312	external encapsulating structure	0	0	0	1
GO:0031012	extracellular matrix	1	1	1	1
GO:0031090	organelle membrane	5	5	5	5
GO:0031224	intrinsic component of membrane	7	7	8	7
GO:0031975	envelope	1	1	1	1
GO:0032993	protein-DNA complex	2	3	3	2
GO:0033061	DNA recombinase mediator complex	1	0	1	1
GO:0034518	RNA cap binding complex	0	1	0	1
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 organelle	11	10	12	11
GO:0043228	non-membrane-bounded organelle	8	9	7	7
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 part	88	85	87	85
GO:0044449	contractile fiber part	0	0	1	1
GO:0044455	mitochondrial membrane part	4	4	2	2
GO:0044459	plasma membrane part	9	9	10	8
GO:0044461	bacterial-type flagellum part	3	1	0	0
GO:0044462	external encapsulating structure part	0	0	0	1
GO:0044463	cell projection part	8	5	4	5
GO:0044815	DNA packaging complex	2	2	2	2
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 polymer	1	1	1	2
GO:0120114	Sm-like protein family complex	5	5	5	5
GO:1902494	catalytic complex	38	36	41	37
GO:1990204	oxidoreductase complex	6	5	6	5
GO:1990351	transporter complex	6	5	7	5
GO:1990391	DNA repair complex	1	1	1	1
GO:1990904	ribonucleoprotein complex	17	17	17	17
Molecular function					
GO:0001871	pattern binding	3	3	3	3
GO:0003700	DNA-binding transcription factor activity	2	0	1	0
GO:0003712	transcription coregulator activity	1	2	3	2
GO:0004133	glycogen debranching enzyme activity	2	2	2	2
GO:0005319	lipid transporter activity	2	2	2	2
GO:0005326	neurotransmitter transporter activity	1	1	1	1
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 activity	1	1	1	1
GO:0009975	cyclase activity	1	2	2	2

GO:0016491	oxidoreductase activity	104	104	104	104
GO:0016530	metallochaperone activity	1	0	0	0
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 transporter activity	62	58	58	61
GO:0030234	enzyme regulator activity	20	19	17	18
GO:0030246	carbohydrate binding	4	4	4	5
GO:0030545	receptor regulator activity	0	1	1	1
GO:0032451	demethylase activity	1	1	1	1
GO:0033218	amide binding	5	5	5	5
GO:0036094	small molecule binding	24	24	22	23
GO:0038023	signaling receptor activity	7	7	7	9
GO:0043167	ion binding	35	34	33	33
GO:0044877	protein-containing complex binding	4	4	3	4
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 activity	1	1	1	1

GO:0061783	peptidoglycan muralytic activity	1	1	1	1
GO:0072341	modified amino acid binding	2	2	2	2
GO:0097159	organic cyclic compound binding	46	45	42	40
GO:0097367	carbohydrate derivative binding	10	9	8	8
GO:0140096	catalytic activity, acting on a protein	68	62	62	61
GO:0140097	catalytic activity, acting on DNA	25	21	25	24
GO:0140098	catalytic activity, acting on RNA	55	56	54	57
GO:1901363	heterocyclic compound binding	46	45	42	40
GO:1901567	fatty acid derivative binding	1	1	1	1
GO:1901681	sulfur compound binding	3	3	3	3

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

GO accession	GO terms	Core	Softcore	Pan
Biological processes				
GO:0002376	immune system process	2	2	3
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 organismal process	1	1	3
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 process	3	4	3
GO:0065007	biological regulation	64	56	78
GO:0071840	cellular component organization or biogenesis	53	54	56
GO:0098754	detoxification	1	1	1
Cellular components				
GO:0016020	membrane	7	9	10
GO:0030054	cell junction	0	0	1
GO:0031974	membrane-enclosed lumen	3	2	3
GO:0032991	protein-containing complex	109	93	119
GO:0043226	organelle	15	18	19
GO:0044421	extracellular region part	4	3	3
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 complex	1	1	2
Molecular function				
GO:0003824	catalytic activity	472	449	476
GO:0005198	structural molecule activity	4	4	5
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 activity	5	5	10
GO:0098772	molecular function regulator	18	14	19
GO:0140104	molecular carrier activity	0	0	1
GO:0140110	transcription regulator activity	2	5	7

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 accession	GO terms	Core	Softcore	Pan
Biological processes				
GO:0000075	cell cycle checkpoint	1	2	2
GO:0002252	immune effector process	1	1	2

GO:0003008	system process	0	0	2
GO:0006457	protein folding	2	0	1
GO:0006807	nitrogen compound metabolic process	227	215	228
GO:0006928	movement of cell or subcellular component	4	5	6
GO:0006950	response to stress	14	14	16
GO:0006955	immune response	0	0	1
GO:0007017	microtubule-based process	4	9	8
GO:0007049	cell cycle	1	1	1
GO:0007059	chromosome segregation	0	2	2
GO:0007154	cell communication	3	2	3
GO:0007155	cell adhesion	0	0	2
GO:0007163	establishment or maintenance of cell polarity	0	1	1
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 stimulus	4	4	5
GO:0009607	response to biotic stimulus	1	2	1
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 interaction between organisms	2	2	2

GO:0048856	anatomical structure development	3	1	1
GO:0048869	cellular developmental process	1	1	0
GO:0048870	cell motility	0	0	2
GO:0050789	regulation of biological process	52	47	66
GO:0051234	establishment of localization	62	71	73
GO:0051235	maintenance of location	1	2	2
GO:0051606	detection of stimulus	1	0	2
GO:0051641	cellular localization	15	16	16
GO:0051716	cellular response to stimulus	10	9	13
GO:0055114	oxidation-reduction process	8	11	13
GO:0061919	process utilizing autophagic mechanism	1	2	2
GO:0065008	regulation of biological quality	15	14	16
GO:0065009	regulation of molecular function	5	6	8
GO:0070085	glycosylation	3	3	3
GO:0070988	demethylation	2	2	2
GO:0071554	cell wall organization or biogenesis	0	1	1
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 process	0	2	2
GO:1990748	cellular detoxification	1	1	1
Cellular components				
GO:0005911	cell-cell junction	0	0	1
GO:0005929	cilium	1	0	2
GO:0008287	protein serine/threonine phosphatase complex	1	1	1
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-bounded organelle	5	8	8
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 membrane part	4	2	3
GO:0044459	plasma membrane part	8	5	8
GO:0044461	bacterial-type flagellum part	0	0	2
GO:0044462	external encapsulating 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 polymer	1	1	2
GO:0120114	Sm-like protein family complex	5	2	4
GO:1902494	catalytic complex	29	26	30
GO:1990204	oxidoreductase complex	6	3	4
GO:1990351	transporter complex	4	3	6
GO:1990391	DNA repair complex	1	1	1

GO:1990904	ribonucleoprotein complex	14	9	16
Molecular function				
GO:0001871	pattern binding	3	3	3
GO:0003700	DNA-binding transcription factor activity	0	0	2
GO:0003712	transcription coregulator activity	1	2	3
GO:0004133	glycogen debranching enzyme activity	2	0	2
GO:0005319	lipid transporter activity	2	2	2
GO:0005326	neurotransmitter transporter activity	0	1	1
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 activity	1	0	1
GO:0009975	cyclase activity	1	2	0
GO:0016491	oxidoreductase activity	92	72	90
GO:0016530	metallochaperone activity	0	0	1
GO:0016740	transferase activity	148	142	144
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 transporter activity	45	49	54
GO:0030234	enzyme regulator activity	14	11	15
GO:0030246	carbohydrate binding	4	5	4
GO:0030545	receptor regulator activity	1	1	1
GO:0032451	demethylase activity	1	1	1
GO:0033218	amide binding	4	4	5
GO:0036094	small molecule binding	21	21	22
GO:0038023	signaling receptor activity	4	5	10
GO:0043167	ion binding	29	30	32
GO:0044877	protein-containing complex binding	4	2	3
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 activity	1	1	1
GO:0061783	peptidoglycan muralytic activity	0	1	1
GO:0072341	modified amino acid binding	1	2	2
GO:0097159	organic cyclic compound binding	36	42	39
GO:0097367	carbohydrate derivative binding	8	8	8
GO:0140096	catalytic activity, acting on a protein	54	50	55

GO:0140097	catalytic activity, acting on DNA	11	21	22
GO:0140098	catalytic activity, acting on RNA	37	48	47
GO:1901363	heterocyclic compound binding	36	42	39
GO:1901567	fatty acid derivative binding	1	1	1
GO:1901681	sulfur compound binding	3	3	3

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

Cluster ID	*G. australes*	*G. carpenteri*	*G. lapillus*	*G. polynesiensis*	*G. holmesii*	**Total 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_UTSMER9A3_Gambierdiscus-carpenteri_DN15967_c2_g1_i2.p1.faa	0	3	0	1	0	4
21082_MMETSP0766_Gambierdiscus-australes_DN32692_c0_g1_i1.p1.faa	0	1	0	2	1	4
195242_UTSMER9A3_Gambierdiscus-carpenteri_DN17326_c2_g5_i1.p1.faa	0	1	1	1	1	4
83891_UTSMER9A3_Gambierdiscus-carpenteri_DN13035_c1_g4_i1.p1.faa	0	1	1	1	1	4
99486_UTSMER9A3_Gambierdiscus-carpenteri_DN13588_c0_g3_i1.p1.faa	0	1	1	1	1	4
328911_HG4_Gambierdiscus-lapillus_DN41464_c0_g1_i1.p1.faa	0	0	1	3	0	4
643864_HG5_Gambierdiscus-silvae_DN47931_c1_g3_i1.p2.faa	0	0	0	0	4	4

186957_UTSMER9A3_Gambierdiscus-carpenteri_DN16979_c3_g3_i1.p1.faa	0	1	1	0	3
193820_UTSMER9A3_Gambierdiscus-carpenteri_DN17268_c1_g8_i4.p1.faa	0	1	1	0	3
147284_UTSMER9A3_Gambierdiscus-carpenteri_DN15408_c1_g3_i2.p1.faa	0	1	1	0	3
116539_UTSMER9A3_Gambierdiscus-carpenteri_DN14227_c2_g1_i4.p1.faa	0	2	0	0	3
242595_UTSMER9A3_Gambierdiscus-carpenteri_DN9176_c0_g1_i3.p1.faa	0	2	0	0	3
524928_CG150_Gambierdiscus-polynesiensis_DN43543_c1_g1_i1.p1.faa	0	0	3	0	3
1040_MMETSP0766_Gambierdiscus-australes_DN11947_c0_g1_i1.p1.faa	0	0	0	2	3
38402_MMETSP0766_Gambierdiscus-australes_DN41494_c1_g1_i3.p1.faa	0	1	0	0	3
154624_UTSMER9A3_Gambierdiscus-carpenteri_DN15679_c0_g6_i1.p1.faa	0	0	0	0	2
63665_UTSMER9A3_Gambierdiscus-carpenteri_DN10182_c0_g1_i2.p1.faa	0	0	0	0	2
205876_UTSMER9A3_Gambierdiscus-carpenteri_DN17803_c0_g4_i1.p1.faa	0	0	0	0	2
224239_UTSMER9A3_Gambierdiscus-carpenteri_DN18618_c3_g6_i1.p1.faa	0	0	0	0	2
196786_UTSMER9A3_Gambierdiscus-carpenteri_DN17387_c2_g2_i1.p1.faa	0	0	1	0	2
131133_UTSMER9A3_Gambierdiscus-carpenteri_DN14782_c2_g4_i3.p1.faa	0	0	0	1	2
19133_MMETSP0766_Gambierdiscus-australes_DN30780_c0_g2_i1.p1.faa	0	0	0	0	2

37007_MMETSP0766_Gambierdiscus-australes_DN41205_c1_g7_i1.p1.faa	1	0	0	0	0	2
424979_CG150_Gambierdiscus-polynesiensis_DN34166_c0_g9_i1.p1.faa	0	0	2	0	0	2
358554_CG150_Gambierdiscus-polynesiensis_DN15070_c0_g1_i1.p2.faa	0	0	2	0	0	2
408901_CG150_Gambierdiscus-polynesiensis_DN32288_c2_g1_i1.p1.faa	0	0	2	0	0	2
479997_CG150_Gambierdiscus-polynesiensis_DN39607_c0_g2_i1.p1.faa	0	0	1	1	0	2
485470_CG150_Gambierdiscus-polynesiensis_DN40097_c0_g1_i2.p1.faa	0	0	1	1	0	2
258909_HG4_CG_Gambierdiscus-lapillus_DN22432_c0_g1_i2.p1.faa	0	0	1	1	0	2
263811_HG4_CG_Gambierdiscus-lapillus_DN25138_c0_g1_i1.p1.faa	0	1	0	1	0	2
319034_HG4_CG_Gambierdiscus-lapillus_DN40675_c3_g1_i2.p1.faa	0	1	0	1	0	2
319505_HG4_CG_Gambierdiscus-lapillus_DN40711_c1_g8_i1.p1.faa	0	1	0	1	0	2
1041_MMETSP0766_Gambierdiscus-australes_DN11947_c0_g2_i1.p1.faa	0	0	0	1	0	2
27066_MMETSP0766_Gambierdiscus-australes_DN36729_c0_g1_i1.p2.faa	0	0	0	1	0	2
274389_HG4_CG_Gambierdiscus-lapillus_DN30113_c0_g1_i2.p1.faa	0	2	0	0	0	2
46553_MMETSP0766_Gambierdiscus-australes_DN42196_c9_g4_i1.p1.faa	2	0	0	0	0	2
148669_UTSMER9A3_Gambierdiscus-carpenteri_DN15462_c1_g7_i1.p1.faa	1	0	0	0	0	1

234513_UTSM0ER9A3_Gambierdiscus-carpenteri_DN23482_c0_g1_i1.p1.faa	0	0	0	1
63664_UTSMER9A3_Gambierdiscus-carpenteri_DN10182_c0_g1_i1.p1.faa	0	0	0	1
72166_UTSMER9A3_Gambierdiscus-carpenteri_DN1258_c0_g1_i1.p1.faa	0	0	0	1
210660_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18011_c6_g4_i1.p1.faa	0	0	0	1
88291_UTSMER9A3_Gambierdiscus-carpenteri_DN13188_c2_g8_i2.p2.faa	0	0	0	1
235070_UTSM0ER9A3_Gambierdiscus-carpenteri_DN25711_c0_g1_i1.p2.faa	0	0	0	1
236919_UTSM0ER9A3_Gambierdiscus-carpenteri_DN33286_c0_g1_i1.p1.faa	0	0	0	1
234708_UTSM0ER9A3_Gambierdiscus-carpenteri_DN24051_c0_g1_i1.p1.faa	0	0	0	1
75892_UTSMER9A3_Gambierdiscus-carpenteri_DN12749_c1_g2_i3.p1.faa	0	0	0	1
207498_UTSM0ER9A3_Gambierdiscus-carpenteri_DN17871_c4_g9_i1.p1.faa	0	0	0	1
234298_UTSM0ER9A3_Gambierdiscus-carpenteri_DN22896_c0_g1_i1.p1.faa	0	0	0	1
84448_UTSMER9A3_Gambierdiscus-carpenteri_DN13053_c3_g3_i4.p1.faa	0	0	0	1
104611_UTSM0ER9A3_Gambierdiscus-carpenteri_DN13776_c4_g7_i1.p1.faa	0	0	0	1
242597_UTSM0ER9A3_Gambierdiscus-carpenteri_DN9176_c0_g2_i2.p2.faa	0	0	0	1
233698_UTSM0ER9A3_Gambierdiscus-carpenteri_DN2009_c0_g1_i1.p1.faa	0	0	0	1

115505_UTSM0ER9A3_Gambierdiscus-carpenteri_DN14189_c2_g12_i1.p1.faa	0	0	0	1
238946_UTSM0ER9A3_Gambierdiscus-carpenteri_DN4887_c0_g1_i1.p1.faa	0	0	0	1
208524_UTSM0ER9A3_Gambierdiscus-carpenteri_DN17914_c1_g3_i4.p1.faa	0	0	0	1
131131_UTSM0ER9A3_Gambierdiscus-carpenteri_DN14782_c2_g4_i1.p1.faa	0	0	0	1
215621_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18221_c2_g6_i3.p1.faa	0	0	0	1
225926_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18701_c1_g3_i2.p1.faa	0	0	0	1
239297_UTSM0ER9A3_Gambierdiscus-carpenteri_DN5390_c0_g1_i1.p1.faa	0	0	0	1
233616_UTSM0ER9A3_Gambierdiscus-carpenteri_DN19857_c0_g1_i1.p1.faa	0	0	0	1
208525_UTSM0ER9A3_Gambierdiscus-carpenteri_DN17914_c1_g3_i5.p2.faa	0	0	0	1
236171_UTSM0ER9A3_Gambierdiscus-carpenteri_DN30145_c0_g1_i1.p1.faa	0	0	0	1
241217_UTSM0ER9A3_Gambierdiscus-carpenteri_DN7872_c0_g1_i1.p1.faa	0	0	0	1
212813_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18098_c3_g3_i2.p1.faa	0	0	0	1
147705_UTSM0ER9A3_Gambierdiscus-carpenteri_DN15422_c1_g3_i1.p1.faa	0	0	0	1
242594_UTSM0ER9A3_Gambierdiscus-carpenteri_DN9176_c0_g1_i2.p1.faa	0	0	0	1
86631_UTSM0ER9A3_Gambierdiscus-carpenteri_DN13131_c1_g1_i1.p1.faa	0	0	0	1

238247_UTSM0ER9A3_Gambierdiscus-carpenteri_DN38343_c0_g1_i1.p1.faa			0	0	0	1
212812_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18098_c3_g3_i1.p1.faa			0	0	0	1
211703_UTSM0ER9A3_Gambierdiscus-carpenteri_DN18052_c3_g5_i1.p1.faa			0	0	0	1
239230_UTSM0ER9A3_Gambierdiscus-carpenteri_DN5288_c0_g1_i1.p1.faa			0	0	0	1
103957_UTSM0ER9A3_Gambierdiscus-carpenteri_DN13754_c3_g2_i4.p1.faa			0	0	0	1
462243_CG150Gambierdiscus-polynesiensis_DN37930_c0_g1_i2.p1.faa		0	1	0	1	
355979_CG150Gambierdiscus-polynesiensis_DN10471_c0_g1_i1.p1.faa		0	1	0	1	
524904_CG150Gambierdiscus-polynesiensis_DN43540_c1_g1_i2.p1.faa		0	1	0	1	
471036_CG150Gambierdiscus-polynesiensis_DN38733_c0_g1_i1.p1.faa		0	1	0	1	
527904_CG150Gambierdiscus-polynesiensis_DN43803_c0_g1_i1.p1.faa		0	1	0	1	
494332_CG150Gambierdiscus-polynesiensis_DN40908_c1_g1_i1.p1.faa		0	1	0	1	
475327_CG150Gambierdiscus-polynesiensis_DN39159_c1_g1_i1.p1.faa		0	1	0	1	
446377_CG150Gambierdiscus-polynesiensis_DN36357_c3_g7_i1.p1.faa		0	1	0	1	
415511_CG150Gambierdiscus-polynesiensis_DN33112_c0_g1_i3.p1.faa		0	1	0	1	
524930_CG150Gambierdiscus-polynesiensis_DN43543_c1_g1_i4.p1.faa		0	1	0	1	

500254_CG150	Gambierdiscus-polynesiensis_DN41444_c1_g3_i1.p1.faa	0	0	1	0	1
408903_CG150	Gambierdiscus-polynesiensis_DN32288_c3_g1_i1.p1.faa	0	0	1	0	1
211708_UTSM	ER9A3_Gambierdiscus-carpenteri_DN18052_c3_g5_i7.p1.faa	0	0	1	0	1
524905_CG150	Gambierdiscus-polynesiensis_DN43540_c1_g1_i3.p1.faa	0	0	1	0	1
528784_CG150	Gambierdiscus-polynesiensis_DN47453_c0_g1_i1.p3.faa	0	0	1	0	1
528223_CG150	Gambierdiscus-polynesiensis_DN44935_c0_g1_i1.p2.faa	0	0	1	0	1
362866_CG150	Gambierdiscus-polynesiensis_DN18821_c0_g1_i1.p1.faa	0	0	1	0	1
408898_CG150	Gambierdiscus-polynesiensis_DN32288_c1_g1_i1.p1.faa	0	0	1	0	1
473656_CG150	Gambierdiscus-polynesiensis_DN39000_c2_g2_i1.p1.faa	0	0	1	0	1
505619_CG150	Gambierdiscus-polynesiensis_DN41913_c1_g3_i1.p2.faa	0	0	1	0	1
357110_CG150	Gambierdiscus-polynesiensis_DN13123_c0_g1_i2.p2.faa	0	0	1	0	1
529123_CG150	Gambierdiscus-polynesiensis_DN48937_c0_g1_i1.p1.faa	0	0	1	0	1
419597_CG150	Gambierdiscus-polynesiensis_DN33575_c2_g1_i1.p1.faa	0	0	1	0	1
486622_CG150	Gambierdiscus-polynesiensis_DN40207_c2_g2_i2.p1.faa	0	0	1	0	1
518712_CG150	Gambierdiscus-polynesiensis_DN43045_c0_g2_i6.p1.faa	0	0	1	0	1

505617_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN41913_c1_g2_i1.p1.faa						
419857_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN33604_c1_g1_i1.p1.faa						
319033_HG4_C	Gambierdiscus-	0	0	1	0	1
lapillus_DN40675_c3_g1_i1.p1.faa						
505612_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN41913_c0_g1_i1.p1.faa						
505621_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN41913_c1_g5_i1.p2.faa						
368243_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN21805_c0_g1_i1.p1.faa						
531066_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN7198_c0_g1_i1.p1.faa						
411779_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN32643_c5_g2_i3.p2.faa						
529709_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN51840_c0_g1_i1.p1.faa						
424815_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN34144_c0_g1_i6.p1.faa						
388829_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN29147_c0_g1_i1.p1.faa						
528991_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN4849_c0_g1_i1.p2.faa						
529886_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN52795_c0_g1_i1.p1.faa						
517572_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN42942_c0_g1_i1.p1.faa						
162319_UTSM	ER9A3_Gambierdiscus-	0	0	1	0	1
carpenteri_DN15967_c2_g1_i1.p1.faa						

486374_CG150	Gambierdiscus-polynesiensis_DN40177_c0_g2_i3.p1.faa	0	0	1	0	1
424977_CG150	Gambierdiscus-polynesiensis_DN34166_c0_g6_i1.p2.faa	0	0	1	0	1
480000_CG150	Gambierdiscus-polynesiensis_DN39607_c0_g2_i4.p1.faa	0	0	1	0	1
524933_CG150	Gambierdiscus-polynesiensis_DN43543_c1_g1_i7.p1.faa	0	0	1	0	1
529340_CG150	Gambierdiscus-polynesiensis_DN50363_c0_g1_i1.p1.faa	0	0	1	0	1
382787_CG150	Gambierdiscus-polynesiensis_DN27509_c0_g1_i1.p1.faa	0	0	1	0	1
455767_CG150	Gambierdiscus-polynesiensis_DN37290_c0_g4_i1.p1.faa	0	0	1	0	1
454667_CG150	Gambierdiscus-polynesiensis_DN37192_c1_g3_i1.p1.faa	0	0	1	0	1
505616_CG150	Gambierdiscus-polynesiensis_DN41913_c1_g1_i3.p1.faa	0	0	1	0	1
408904_CG150	Gambierdiscus-polynesiensis_DN32288_c3_g2_i1.p1.faa	0	0	1	0	1
519735_CG150	Gambierdiscus-polynesiensis_DN43127_c3_g5_i1.p1.faa	0	0	1	0	1
524932_CG150	Gambierdiscus-polynesiensis_DN43543_c1_g1_i6.p1.faa	0	0	1	0	1
419608_CG150	Gambierdiscus-polynesiensis_DN33575_c2_g2_i1.p1.faa	0	0	1	0	1
489214_CG150	Gambierdiscus-polynesiensis_DN40447_c0_g1_i2.p1.faa	0	0	1	0	1
407098_CG150	Gambierdiscus-polynesiensis_DN32057_c0_g1_i2.p1.faa	0	0	1	0	1

486620_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN40207_c2_g1_i2.p2.faa						
529847_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN52688_c0_g1_i1.p1.faa						
355910_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN1036_c0_g1_i1.p2.faa						
419599_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN33575_c2_g1_i11.p1.faa						
368244_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN21805_c0_g2_i1.p1.faa						
528301_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN45312_c0_g1_i1.p1.faa						
431157_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN34812_c2_g1_i1.p1.faa						
429838_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN3467_c0_g1_i1.p1.faa						
485799_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN40132_c0_g3_i1.p1.faa						
449384_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN36673_c0_g1_i3.p1.faa						
530384_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN55090_c0_g1_i1.p1.faa						
357109_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN13123_c0_g1_i1.p2.faa						
466543_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN38313_c1_g3_i1.p1.faa						
367731_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN21547_c0_g1_i1.p1.faa						
438506_CG150	Gambierdiscus-	0	0	1	0	1
polynesiensis_DN35575_c0_g1_i7.p1.faa						

491823_CG150	Gambierdiscus-polynesiensis_DN40690_c4_g5_i2.p1.faa	0	0	1	0	1
530249_CG150	Gambierdiscus-polynesiensis_DN54681_c0_g1_i1.p1.faa	0	0	1	0	1
661643_HG5_0	Gambierdiscus-silvae_DN57114_c0_g1_i1.p1.faa	0	0	0	1	1
601478_HG5_0	Gambierdiscus-silvae_DN43780_c7_g8_i1.p1.faa	0	0	0	1	1
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596728_HG5_0 silvae_DN43120_c1_g4_i4.p1.faa	Gambierdiscus	0	0	1	1
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16842_MMETSP0766_Gambierdiscus-australes_DN28731_c0_g3_i1.p2.faa	0	0	0	1

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