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VitisGDB: The Multifunctional Database for Grapevine Breeding and Genetics

Grapevine cultivation has been gaining commercial popularity in many parts of the world due to the high yield and versatility of this horticultural crop. A recent survey from the International Organization of Vine and Wine (OIV) estimated that the global area under vine cultivation in 2018 was about 7.4 million hectares and that the world production of grapes was about 77.8 million tons in total (OIV, 2019). The majority of the global grape yield is used for producing wines, fresh fruit, and raisins, bringing in annual revenue of billions of US dollars (Alston and Sambucci, 2019). In addition to its economic value, the grapevine is also a useful model for the study of the genetic basis of clonality, fruit development, sex determination, grafting, evolution, and domestication (This et al., 2006). Furthermore, for many countries in the world traditional viniculture and viticulture are important emblems of cultural identity. All these factors have made grapevine one of the most heavily invested plants in horticultural research.

The rise of genome-sequencing technologies has facilitated the release of reference-grade genetic codes and individual-level genetic variations for many grapevine species and cultivars (Canaguier et al., 2017; Zhou et al., 2017; Roach et al., 2018; Girollet et al., 2019; Liang et al., 2019; Minio et al., 2019; Vondras et al., 2019). Despite the increasing genomic data, a reliable platform for comparing and mining Vitis genomic information is not available. To fill this gap, we have developed VitisGDB, an online genus-level multifunctional genomics database for grapevine (Figure 1 and Supplemental Note; http:// vitisgdb.ynau.edu.cn/). VitisGDB aggregates genetic information for 50 out of 60 extant Vitis species, provides the results with visualization of a series of common genetic analyses, and implements easy-to-use bioinformatic tools to enable the investigation of economically important traits for breeding new grapevine cultivars.

The framework of VitisGDB was constructed with MySQL, ThinkPHP, and FastAdmin (Figure 1 and Supplemental Note) to allow for easier data organization and a user-friendly interface. Four main modules, namely species, germplasm, phenotype, and gene (Figure 1), were created for the effective categorization and access of aggregated grapevine data. In brief, the species module provides easy retrieval of information for one European Vitis species (two subspecies), 19 North American Vitis Species, 26 East Asia Vitis Species, and three species from other genera (Supplemental Figure 1). The main web page for each Vitis species starts with a species profile information section, which includes Latin name, chromosome geographical distribution, and morphological number, description. A representative picture (if publicly available) is also provided to facilitate taxonomic identification of the species. The second section lists the statistics of all available reference genome assemblies, by which the quality of the assemblies (contig N50, scaffold N50, and BUSCO value) can

be compared. The following section details a table of sequenced germplasm with extensive ID information. The final section presents interactive graphs of the phylogenetic tree and the population genetic analyses. The phylogenetic tree shows a clear classification of major grapevine groups, and the accession label shows detailed information for each grapevine. Both the scatterplot of principal component analysis and the bar plot of ADMIXTURE analysis can be zoomed in and out for clarity. The summary statistics of agronomic trait values in the form of box-plot distributions are also presented. Finally, users have access to species-related literature that is periodically updated.

The germplasm module includes the passport data, wholegenome sequences, and published phenotypic data for 1641 *Vitis* accessions, which are reported by various resequencing projects. To resolve the issue that a single cultivar may have different names, the genetic background of each accession was determined using SNP data and cross-verified with the VIVC database. Consequently, accessions with the same genetic background are grouped under the same prime name, whereas 28 accessions that might be misidentified are highlighted with the inferred taxa in the germplasm module and the phylogenetic tree section under species module.

The phenotype module indexes numeric values or categorical values for a total of 45 grapevine phenotypic trait data from 1461 accessions. For each trait, the descriptor includes trait name, trait unit, OIV code, scale, and a brief summary of how the trait value was obtained. All phenotypic values are presented in a table with a histogram plot showing their distribution.

Gene annotation results for three chromosome-level reference genomes are integrated in the gene module. A total of 104 454 genes are curated. The web page for each gene sequentially lists summary information (gene locus ID, gene symbol, gene type, position, and transcript number). The gene structure can be viewed in an embedded JBrowse. The coding sequence (CDS) of the gene and the amino acid sequence of the protein product are provided. The identified SNPs around and within the gene are also listed to facilitate marker selection for functional verification analysis. The expression level of the gene is presented in a heatmap for easy visualization.

In addition to the main modules, VitisGDB contains a total of 25 integrated tools and external databases devoted to *Vitis* genetic research (Supplemental Figure 2). For instance, the BLAST tool is incorporated into a stand-alone web page, where 19 genome assemblies, seven CDS sequence databases, and seven protein

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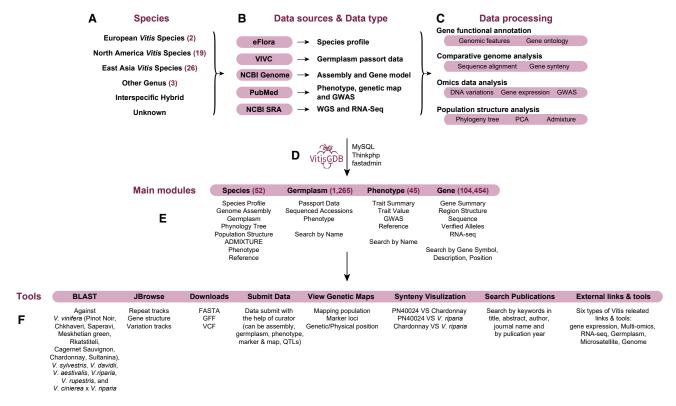


Figure 1. Schematic Illustration of VitisGDB

(A) Species information. Information for 50 species in six groups was collected. Highlighted numbers in parentheses indicate number of species under each group.

(B) Data sources and data type. Data sources include web sites such as eFlora, VIVC, and the public database NCBI, as well as literature. Data type includes information about species, germplasm, genome assembly, gene annotation, phenotypic trait values, genetic maps, resequencing data, and RNA-sequencing data.

(C) Summary of data-processing methods.

(D) Framework of VitisGDB. All data are stored in MySQL. Thinkphp and FastAdmin are used for front-end and back-end data management.

(E) Main modules. Highlighted numbers in parentheses indicate the number of corresponding entries.

(F) Overview of the integrated tools in VitisGDB with main functions listed below.

sequence databases are available for query of orthologous gene candidates. The input can be either plain text or a fasta sequence file. The alignment result (available in eight styles) opens up in a new page, detailing the overall alignment score, query length, and similarity between the query and subject sequences. The BLAST result allows secondary filtering, and the final subjects can be downloaded in HTML format.

JBrowse is an efficient visualization tool, which facilitates the viewing of gene models, CDS, heterozygous SNPs, and RNA sequencing data, each presented in a different color, in the context of the genomic region. At the moment, all available *Vitis* genomes and gene models are incorporated into JBrowse.

The JavaScript-based tool SynVisio is implemented to show the synteny relationships of three pairs of chromosome-level reference genomes (PN40024 versus Chardonnay, PN40024 versus *Vitis riparia*, and Chardonnay versus *V. riparia*). The visualization includes a hive plot indicating synteny between chromosomes, a dot plot indicating collinearity between two species/ cultivars, and a scatterplot indicating identified signal strength. The threshold for displaying results in the hive plot and dot plot can be selected by dragging the little circle on the value bar

from the min to the max (above the dotted line at the lower left corner).

To date, three grapevine genetic maps are available, covering a total of 70 832 marker loci on the genome. For a selected genetic map, a heat plot shows the density of loci along the chromosomes and a corresponding table provides basic information about the mapping population and the genetic map. Doubleclicking on the heat plot will zero in on a chromosome of interest on a new web page. The chromosome can be sized with the pointer to view regions in finer detail and show details for each locus with a hyperlink to JBrowse.

To allow personalized usage and analyses of the data, we have built a "Download" web page for all datasets available to the public. These include genome assembly sequences, annotation results, and genomic variations in FASTA, GFF, and VCF format, respectively. Considering the large size of the raw data for *de novo* assembly, resequencing projects, and RNA sequencing, we provide the NCBI BioProject ID and BioSample ID as well as the corresponding links on the web page. We have also imported the metadata for all *Vitis*-related publications from NCBI into VitisGDB for quick searching. Please cite this article in press as: Dong et al., VitisGDB: The Multifunctional Database for Grapevine Breeding and Genetics, Molecular Plant (2020), https://doi.org/10.1016/j.molp.2020.05.002

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In summary, VitisGDB provides the most comprehensive view of *Vitis* genomic data to date and will be a valuable platform for studies on *Vitis* functional genomics and agronomic improvement. With the goal of becoming a community-built platform dedicated to making research results on grapevine broadly available, VitisGDB accepts the submission of all types of grapevine genetic data via the "Submit Data" page. VitisGBD will be continuously updated as genomic data from ongoing sequencing projects become available. New tools and analysis for transposable elements, non-coding RNAs, and environmental data will be added, so that VitisGDB will provide long-term support to the grapevine research community.

SUPPLEMENTAL INFORMATION

Supplemental Information can be found online at Molecular Plant Online.

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Supplemental Information

VitisGDB: The Multifunctional Database for Grapevine Breeding and

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1 SUPPLEMENTAL INFORMATION

2 VitisGDB: the Multifunctional Database for Grapevine Breeding and Genetics

- 3 Dong, X., Chen, W., et al.
- 4

5 Supplementary Note:

6 Status quo of existing Vitis databases

7 Database is a source of information where users are able to access and organize knowledge of

8 a common theme. Modern genome databases for plants (i.e. Rosacea

9 https://www.rosaceae.org/, maize https://www.maizegdb.org/, and cucurbit

10 http://cucurbitgenomics.org/) often incorporate bioinformatic tools and even germplasm

11 services, thereby, enabling the users to perform multiple tasks with ease (Martinez, 2016).

12 Currently, there are many Vitis databases where only descriptive information (i.e. species

13 name, country of origin, cultivar names, berry color, and usage etc.) and/or simple genetic

14 information (i.e. EST and SSR) for grapevine accessions are curated. These include the Vitis

15 International Variety Catalogue (VIVC, http://www.vivc.de/), the European Vitis database

16 (http://www.eu-vitis.de/index.php), the Armenian Vitis Database (http://www.vitis.am/eng),

17 the Bulgarian Vitis Database (http://www.bulvitis-db.com/) and so on. The conventional

18 genomic database such as the National Center for Biotechnology Information (NCBI,

19 https://www.ncbi.nlm.nih.gov/) and Ensembl (http://ensembl.gramene.org) does provide

20 access to all genomic data of published grapevines, but it is not a specialized platform for

21 grapevine genetics and breeding. There are also other databases and websites devoted to a

22 single aspect of grapevine genetic information. They could be grouped into several major

23 categories: (1) Gene expression browser (i.e. VTCdb; http://vtcdb.adelaide.edu.au/Home.aspx

and Grape eFP Browser; http://bar.utoronto.ca/efp_grape/cgi-bin/efpWeb.cgi). (2) Molecular

25 pathway database (i.e. VitisNet; https://www.sdstate.edu/vitisnet-molecular-networks-

26 grapevine). (3) Transcriptome database (http://www.grapeworld.cn/gt/index.html). (4)

27 Grape sRNA atlas (https://mpss.danforthcenter.org/dbs/index.php?SITE=grape_sRNA_atlas).

- 28 (5) Microsatellite database like SVMD (http://www1.unine.ch/svmd/) and GMC
- 29 (http://meteo.iasma.it/genetica/gmc.html). (6) Grapevine Genome database (i.e. UGRI;
- 30 https://urgi.versailles.inra.fr/Species/Vitis and grape genome database;
- 31 http://genomes.cribi.unipd.it/grape/index.php).
- 32

33 All data sources used in the development of VitisGDB

- 34 (1) <u>Genome Assemblies</u>. A total of 22 Vitis genome assemblies were included in the
- 35 VitisGDB. The genome assemblies of eight cultivated grapevines (Chkhaveri, Saperavi,
- 36 Meskhetian green, Rkatsiteli (Tabidze et al., 2017), Chardonnay clone I10V1 (Roach et al.,
- 37 2018), Tannat clone UY11 (Da Silva et al., 2013), Sultanina (Di Genova et al., 2014),
- 38 Zinfandel clone 03 (Vondras et al., 2019)), two wild species (*V. aestivalis* cv. Norton and *V.*
- 39 riparia cv. Gloire de Montpellier (Girollet et al., 2019)), and one hybrid cultivar (V. cinerea
- $40 \times V.$ *riparia* cv. Boerner) were collected from the NCBI assembly database
- 41 (https://www.ncbi.nlm.nih.gov/assembly/), and the genome assembly of PN40024 12X.v2
- 42 (Jaillon et al., 2007) was downloaded from Ensembl
- 43 (http://ensembl.gramene.org/Vitis_vinifera/Info/Index). Six genome assemblies were
- 44 acquired from private websites, including Cabernet Sauvignon clone 08 (Chin et al., 2016)
- 45 and Carmenere clone 02 (Minio et al., 2019) from the Cantu Lab webpage
- 46 (https://cantulab.github.io/data.html), Chardonnay clone FPS 04 (Zhou et al., 2019) from
- 47 Zenodo (https://zenodo.org/record/3337377#.XhQ49fkzZaR), and three other assemblies of
- 48 Sultanina (Patel et al., 2018) from Open PRAIRIE of South Dakota State University
- 49 (https://openprairie.sdstate.edu/vitis_vinifera_sultanina/1/). Additionally, our lab sequenced
- and assembled the genomes of one wild European grape (V. vinifera subsp. sylvestris), two
- 51 North America Species (V. rupestris and V. riparia) and one East Asia Species (V. davidii).
- 52 These unpublished draft assemblies will be first released in VitisGDB.

(2) <u>Whole-genome Resequencing Data.</u> Raw sequencing reads of 27 grapevine accessions
from Zhou et al. (2017) were downloaded from the NCBI SRA database. The sequencing
reads of 472 *Vitis* accessions from our group were also deposited in VitisGDB.
(3) <u>RNA-Seq Data.</u> Raw sequencing reads of 150 SRA experiments from BioProject
PRJNA386889 (Fasoli et al., 2018) were downloaded from the NCBI SRA database, which
include 17 time points of berry development from fruit set to maturity in Pinot Noir and

59 Cabernet Sauvignon.

60 (4) *Phenotypic Data*. Profile information of the *Vitis* species and cultivars was gathered from

61 eFlora (http://efloras.org/) and the passport data of all *Vitis* accessions were verified against

62 the VIVC database (http://www.vivc.de/). Phenotypic data and genome-wide association

63 analysis results were obtained partly from our lab (Liang et al., 2019), and partly from

64 publications (Guo et al., 2019; Laucou et al., 2018).

65

66 Genomic Data processing

Genome collinearity analysis between the chromosome-level assemblies (PN40024 (Jaillon et
al., 2007), Chardonnay clone FPS04 (Zhou et al., 2019), and *V. riparia* cv. Gloire de
Montpellier (Girollet et al., 2019)) was performed using MCScanX (Wang et al., 2012) with
default settings.

71

72 Whole-genome resequencing reads were mapped to the *Vitis vinifera* reference genome

73 (Jaillon et al., 2007) with BWA (Li and Durbin, 2009) using the default parameters. Software

54 SAMtools (Li et al., 2009) was used to convert mapping results into the BAM format, and

then filter unmapped and non-unique reads. The Picard package

76 (http://broadinstitute.github.io/picard/) was used to filter the duplicated reads. For variation

detection, the best practice workflow recommended by Genome Analysis Toolkit (GATK)

78 (McKenna et al., 2010) was applied. SNPs annotation were performed according to the

result using the package ANNOVAR (Wang et al., 2010).

80

81	Based on the detected SNPs, Principal component analysis (PCA) was performed with the
82	software Genome-wide Complex Trait Analysis (GCTA) (Yang et al., 2011). The first three
83	eigenvectors were plotted in an interactive graph. Population structure was analyzed using the
84	ADMIXTURE program (Alexander et al., 2009) with K ranging from 2 to 14 by a block-
85	relaxation algorithm. The whole-genome SNPs were used to construct the ML phylogenetic
86	tree with 100 bootstrap by SNPhylo (Version: 20140701)(Lee et al., 2014).
87	
88	Fastq-dump tool in the SRA Toolkit (v.2.9.6, https://github.com/ncbi/sra-tools) was used to
89	convert .sra files of the transcriptomic data into fastq format. Trimmomatic (v.0.32) (Bolger
90	et al., 2014) was then used to trim the sequencing reads by the following the parameters
91	"ILLUMINACLIP: Trimmomatic-0.32/adapters/TruSeq3-SE.fa:2:30:10 LEADING:3
92	TRAILING:3 SLIDINGWINDOW:4:15". The clean RNA-seq reads were aligned to the V.
93	vinifera genome assembly using TopHat (v. 2.0.10) (Trapnell et al., 2009) with default
94	parameters and the FPKM value was calculated for each protein-coding gene by Cufflinks (v.
95	2.1.1) (Trapnell et al., 2012) using default parameters.
96	
97	Data integration
98	Profile information of Vitis species, sequenced germplasm, genome assemblies, phenotypic
99	traits, and all relevant information of protein-coding genes were sorted into different
100	categories in a MySQL database (https://www.mysql.com). Keywords was curated for the

101 internal query and hyperlink of all information.

102

103 Database construction

104 Since all data were stored and managed by MySQL, ThinkPHP and FastAdmin were used for

105 frontend and backend interactive queries, respectively. All genomic features were visualized

106 in JBrowse (Buels et al., 2016) and its plugins. The BLAST server was supported by

107	Viroblast (Deng et al., 2007). Echarts (https://www.echartsjs.com/), JavaScript and jQuery
108	were implemented to enhance user experience. Phylogeny.IO (Nikola and S, 2019)
109	(https://github.com/oist/phylogeny-io) was used for interactive visualization of the
110	phylogenetic analysis result. QuiGMap (https://github.com/MFlores2021/QuiGMap) and
111	SynVisio (https://github.com/kiranbandi/synvisio) were applied to visualize genetic map and
112	genome synteny analysis, respectively. All functionalities of VitisGDB have been tested in
113	Google Chrome, Apple Safari, and 360 Browser.
114	
115	Publication query criteria
116	By using the query in NCBI PubMed: (((((((((((((trait[Title/Abstract]) OR
117	QTL[Title/Abstract]) OR gene[Title/Abstract]) OR genome[Title/Abstract]) OR
118	map[Title/Abstract]) OR microsatellite[Title/Abstract]) OR annotation[Title/Abstract]) OR
119	EST[Title/Abstract]) OR marker[Title/Abstract]) OR sequence[Title/Abstract]) OR
120	GWAS[Title/Abstract]) AND (((vitis[Title/Abstract]) OR Vitaceae[Title/Abstract]) OR
121	grape[Title/Abstract]), we obtained and imported the metadata for a total of 3,943
122	publications to VitisGDB.
123	
124	Data availability
125	The raw sequencing data sets generated and used in the VitisGDB are available from NCBI
126	under the BioProject accessions PRJNA625615, PRJNA625617, PRJNA625619,
127	PRJNA625621 for V. vinifera subsp. sylvestris, V. rupestris, V. riparia, and V. davidii,
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129	
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Genus	Vitis		
Species	V. vinifera		
Common name	Cultivated grape		
Distribution	Worldwide		
Chromosome number	2n = 38		
Morphological Description			
Hens sprucing to moderatory high (minking, spravely branched hendens har electronics in strends or jamos, and diaphapung 1-5 drawn finds, instruktur, instren is allphily angled, pulsevents, marining angled threatening the strends of the strends of the strends of the strends in the strends of the strends of the strends 2 consections instant, nodes not the basiled Lawres signator smally consections instant, nodes not the standard Lawres in signator strends 2 consections in the strends of the strends of the strends of the consections of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the Barrier usually reliably purgle to markly lacks, strends on a destingt to purgle a strends of the strends of the strends of the strends of the strends Barrier usually reliably purgle to markly lacks, strends on a destingt to purgle a strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends of the strends			



Assertby	Assoribly level	Size(Mb)	Careful Nild(Mb)	Scatfold NS0(Mb)	Gene number	BUSCO(9
w. Pinot Nair clone PN400024 (Rd	Ghr	493.21	0.065	2.07	30434	
w. Pinot Noir clone PN400024 32X.v0)	Ow	486.26		22.39	22863	95.6
v. Pinet Noir clone PN40004 525.v2)	Ow	405.2	0.102	22.39	41163	95.6
w. Chilhaveri	0¥	427.17	0.112	22.7		92
ov. Sapenavi	Ow	427.04	0.112	22.09		92
ov. Maskhatian green	Onr	427.21	0.112	22.71		92
ov. Risatsiteli	OW.	428.6	0.112	22.67		92
v. Cabernet Sauvignon clone 08	C1g	501.42 (P-contigs), 307.78 (Haplotigs)	2.173 (P-covitga), 0.779 (Haplotiga)		55035 (P-contigs), 40444 (Paplotigs)	95.4
x. Chardonney clone 110/1	C1g	490 (P-contign), 378 (Haplotign)	0.036 (P-contigs), 0.318 (Haplotigs)			93.54
x. Chardonnay clone FPS 04	Ow	608	1,24	24.5	38020	93.4

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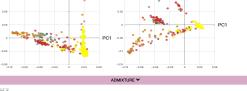
Detabase ID	Prime name	Country of Origin	VIVC
07000000	Patri Kalamak	Afghanistan	4033
9000000	Kandahari Siah	Afghanistan	5960
raveocevor	Naces	Afghanistan	60-63
207000006	Colodan	Afghanistan	26067
Lawoooos	Zana	Albania	13384
10000001	Criola Grande Senjuanina	Argentine	0241
andvoodooz	Moscatual	Argonina	8059
ARGVOCOCOR	Pasiga	Argentina	8964
ARGV000004	Parion	Argantina	9670
andvococos	Big Muscal Soutiess	Argentina	17810

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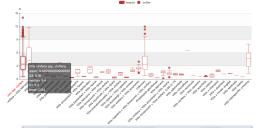
D







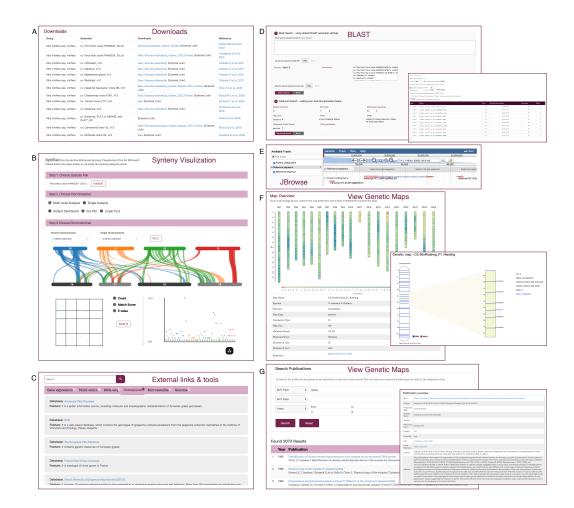
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Reference 🛩						
Author	Title	Journal	Year	PMID		
Amanda M. Vondras et al.	The genomic diversification of clonally propagated grapewines	BioRxiv	2019			
Andrea Minio et al.	Diploid Genome Assembly of the Wine Grape Carménére	G3 (Bethesda)	2019	30923138		
Guo D L, et al.	Genome-wide association study of berry-related traits in grape [Vitis vinifera L.] based on genotyping- try-asquancing markers	Horticulture Research	2019	3060309		
Liang Z, et al.	Whole-genome resequencing of 472 Vitis accessions for grapevine diversity and demographic history analyses	Nature Communications	2019	30887414		
Zhou Y, et al.	The population genetics of structural variants in grapewine domestication.	Nature Plants	2019	31505640		
Laucou V, et al.	Extended diversity analysis of outtivated grapevine Vitis vinifera with 10K genome-wide SNPs	PLoS One	2018	29620600		
Roach MJ , et al.	Population sequencing reveals clonal diversity and ancestral inbreeding in the grapevine cultivar Chardonnay	PLoS Genet	2018	3046900		
Sagar Patel et al.	Comparison of three assembly strategies for a heterozygous seedless grapevine genome assembly	EMC Generalics	2018	29343235		
Canaguier A, et	A new version of the grapevine reference genome assembly (12X,v2) and of its annotation (VCost,v3)	Genomics Data	2017	28971018		

226 Supplementary Figure 1: An example of Species module for V. vinifera ssp. vinifera. (A) 227 Basic species information, which include Latin name, chromosome number, distribution area 228 and description. Figures on two sides are preventive picture and geographic distribution of 229 the sequenced germplasm. (B) Available genome assembly, which listed assembled cultivar 230 and clone, as well as the assembly statistics. (C) Sequenced germplasm, which contains 231 database ID in VitisGDB, prime name, country of origin and ID in VIVC database. (D) 232 Interactive Phenology tree, which includes 497 accessions and their detailed information. 233 Branches are colored by Vitis group. (E) Scatter plot of principle component analysis result, 234 which indicate the species population structure with the corresponding species highlighted in 235 yellow. (F) Bar plot of ADMIXTURE result with K from 2 to 14. Zooming into the detail 236 shows the detail information of accessions of such species. (G) Box plot/bar plot which 237 present the distribution of quantitative/qualitative trait. (H) Related references.

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240 Supplementary Figure 2: User-friendly interfaces for seven integrated tools. (A)

- 241 BLAST; (B) JBrowse; (C) Downloads; (D) View Genetic Maps; (E) Synteny Visualization;
- 242 (F) Search Publications. (G) External Links & Tools.

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