Comprehensive Genome-Wide Classification Reveals That Many Plant-Specific Transcription Factors Evolved in Streptophyte Algae

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Abstract

Plant genomes encode many lineage-specific, unique transcription factors. Expansion of such gene families has been previously found to coincide with the evolution of morphological complexity, although comparative analyses have been hampered by severe sampling bias. Here, we make use of the recently increased availability of plant genomes. We have updated and expanded previous rule sets for domain-based classification of transcription associated proteins (TAPs), comprising transcription factors and transcriptional regulators. The genome-wide annotation of these protein families has been analyzed and made available via the novel TAPscan web interface. We find that many TAP families previously thought to be specific for land plants actually evolved in streptophyte (charophyte) algae; 26 out of 36 TAP family gains are inferred to have occurred in the common ancestor of the Streptophyta (uniting the land plants—Embryophyta—with their closest algal relatives). In contrast, expansions of TAP families were found to occur throughout streptophyte evolution. 17 out of 76 expansion events were found to be common to all land plants and thus probably evolved concomitant with the water-to-land-transition.

Key words: Charophyta, Streptophyta, Embryophyta, evolution, transcription, land plant.

Introduction

Transcriptional regulation is carried out by transcription associated proteins (TAPs), comprising transcription factors (TFs, binding in sequence-specific manner to *cis*-regulatory elements to enhance or repress transcription), transcriptional regulators (TRs, acting as part of the transcription core complex, via unspecific binding, protein–protein interaction or chromatin modification) and putative TAPs (PTs), the role of which needs to be determined (Richardt et al. 2007).

The complexity of transcriptional regulation (as measured by the genomes' potential to encode TAPs, i.e., total number of TAP genes per genome) coincides with the morphological complexity (typically measured by number of cell types) of plants and animals (Levine and Tjian 2003; Lang et al. 2010; de Mendoza et al. 2013; Lang and Rensing 2015). Comparative studies in plants and animals have revealed gains, losses and expansions of key gene families, and demonstrated the unicellular ancestors of plants and animals had already gained much of the families known as important and typical for these lineages (Lang et al. 2010; de Mendoza et al. 2013; de Mendoza et al. 2015; Catarino et al. 2016). The recent initial analysis of data from streptophyte algae (sharing common ancestry with land plants) suggested that the origin of TAPs considered to be specific for land plants needs to be revised (Hori et al. 2014; Delaux et al. 2015; Wang et al. 2015), which we set out to do here by including more data of streptophyte algae and bryophytes than previously available.

Transcription associated proteins, and in particular TFs, are important signaling components and as such often key regulators of developmental progressions. They evolve via duplication, paralog retention and subsequent sub- and neofunctionalization (Rensing 2014), leading to a high abundance and combinatorial complexity of these proteins in the most complex multicellular lineages (that perform embryogenesis)—namely plants and animals (de Mendoza et al. 2013; Lang and Rensing 2015; Rensing 2016).

Many plant TFs have initially been described as regulators of organ development or stress responses of flowering plants. However, by broadening the view to other plants it became clear that, for example LFY, initially described in *Arabidopsis thaliana* as determining the floral fate of meristems and regulating flower patterning, controls the first division of the zygote in the moss *Physcomitrella patens* (Tanahashi et al. 2005). Also, the flowering plant meristem controlling WOX genes have orthologs in moss that are involved in apical stem cell formation (Sakakibara et al. 2014). Such homeodomain (HD) TFs have deep eukaryotic roots and control important developmental progressions, for example, in embryogenesis, in plants, and animals (Hudry et al. 2014; Catarino et al. 2016). The KNOX and BELL subfamilies of plant HD proteins control mating types of green algae (Lee et al. 2008) and evolved into controlling cell fate determination of flowering plant stem cells (Hay and Tsiantis 2010). TF gene regulatory network kernels that were present in the earliest land plants are often modified and coopted during evolution (Pires et al. 2013), and plant TF paralogs are preferentially retained after whole genome duplication (WGD) events (De Bodt et al. 2005; Lang et al. 2010). TRs do not show the same tendency as TFs to expand with complexity, but they are important regulators nevertheless. For example, epigenetic control of important developmental steps like body plan control is maintained via components of the Polycomb Group (PcG) proteins throughout land plants (Mosquna et al. 2009; Okano et al. 2009; Bouyer et al. 2011).

Transcription associated proteins are thus key to understanding development and evolution of plant form and function. Access to reliable, up-to-date classification of TAPs is important, and enables comparative analyses informing our knowledge of plant transcriptional regulation. In a previous study (Lang et al. 2010) we combined rule sets of three studies (Riano-Pachon et al. 2007; Richardt et al. 2007; Guo et al. 2008) to generate the comprehensive TAPscan tool, encompassing sensitive domain-based classification rules for 111 TAP families. Similar approaches were undertaken by other studies, for example, PlnTFDB (Perez-Rodriguez et al. 2010), iTAK (Zheng et al. 2016), or PlantTFDB (Jin et al. 2016). We have now expanded our methodology by switching to HMMER v3, by updating the Hidden Markov Models (HMM) of many of the domains, and by including novel subfamily classification for several families. Moreover, we have included 92 more genomes than were available 7 years ago, dramatically improving taxon sampling. Here, we present an updated comprehensive analysis of TAP evolution of the green lineage as well as the TAPscan v2 web interface (http://plantco.de/tapscan/), including precomputed gene trees. This interface is a successor to PlnTFDB v3.0 (Perez-Rodriguez et al. 2010), encompasses the most comprehensive set of plant TAPs, and represents a novel tool for the plant community to access, screen and download genome-wide TAP annotations.

Materials and Methods

Data Set

In our previous analysis (Lang et al. 2010) no streptophyte algae, no gymnosperms and only a single bryophyte genome were covered. Here, we collected a set of 110 genomes and 13 transcriptomes with the purpose of covering as many major clades as possible within the Viridiplantae (green lineage, table 1 and supplementary table S5, Supplementary Material online), and to close the previous taxonomic holes.

Upgrade to HMMER3 and New PFAM Profiles

The extensive update of HMMER from v2 to v3 included improvements in both sensitivity and run time. With this new version, HMMER abandoned its glocal (global/local) approach, the alignment of a complete model to a subsection of a protein, to exclusively use local alignments. This change made it possible to make use of how much of the respective HMM profile was matched per alignment. This information was implemented in our TAPscan pipeline as a dynamic coverage cutoff aimed to introduce a higher level of strictness to maintain sequence and functional conservation. For our custom-built profiles we set this cutoff to 75% based on manual inspection of the alignments (cf. Results). For the PFAM profiles we calculated the proportion of 100% conservation in each profiles' seed alignment and used this as minimum coverage cutoff (listed in supplementary table S3, Supplementary Material online). Out of the 124 HMM profiles published in 2010 (Lang et al. 2010), 108 had been obtained from the PFAM database (PFAM 23.0) and were again downloaded directly from the PFAM database (PFAM 29.0).

Updating the Custom-Built HMM Profiles

The 16 domains represented by custom-made profiles had to be updated separately. They were first checked against the PFAM database to see if any equivalent profiles could be found, which was true only for NAC/NAM (supplementary table S1, Supplementary Material online). To increase the sequence diversity underlying the HMM profiles we decided to not directly reuse the profile multiple sequence alignments published earlier (Lang et al. 2010), but instead to use the output of these profiles when run against a database of 46 genomes representing 12 diverse groups of organisms (supplementary table S2, Supplementary Material online; 2x animals, 1x bryophyte, 8x chlorophytes, 1× conifer, 9× dicots, 1× lycophyte, 6× fungi, 1× glaucophyte, 4× monocots, 1× charophyte, 7× protoctista [5× nongreen algae, 1× Mycetozoa, 1× Heterolobosea], and 5× rhodophytes).

Table 1
Included Species

Taxonomic Group	**Lang et al. (2010) V1**	**V2 2017**		**Unpublished Genomes**	**Unpublished Transcriptomes**
	Genomes	**Genomes**	**Transcriptomes**		
Angiosperm—Core Eudicots/Core Rosids	7	46	0	1 (*Salix purpurea*)	
Angiosperm—Core Eudicots/Asterids	0	11	0		
Angiosperm—Core Eudicots/Stem Rosids	0	4	0	1 (*Kalanchoe laxiflora*)	
Angiosperm—Stem Eudicots	0	2	0	1 (*Aquilegia coerulea*)	
Angiosperm—Monocots	3	22	0	3 (*Brachypodium stacei, Panicum virgatum, Setaria viridis*)	
Angiosperm—ANA grade (stem angiosperms)	0	1	0		
Sub total angiosperms	**10**	**86**	**0**		
Gymnosperm—Conifer	0	2	1		
Gymnosperm—Ginkgophyte	0	1	0		
Monilophytes—Leptosporangiate	0	2	2	2 (*Azolla filiculoides* and *Salvinia cucullata*)	1 (*Microlepia* cf. *marginata*)
Lycophytes	1	1	0		
Mosses	1	2	2	1 (*Sphagnum fallax*)	
Liverworts	0	1	1		
Sub total nonseed plants and gymnosperms	**2**	**9**	**6**		
Streptophytic Algae—Zygnematales	0	1	2		
Streptophytic Algae—Coleochaetales	0	0	3		
Streptophytic Algae—Charales	0	1	1	1 (*Chara braunii*)	
Streptophytic Algae—Klebsormidiales	0	1	0		
Streptophytic Algae—Chlorokybales	0	0	1		
Green Algae—Chlorophyta	7	13	0	1 (*Dunaliella salina*)	
Sub total algae	**7**	**16**	**7**		
Total	**19**	**111**	**13**		
		124			

Note.—Species are divided into angiosperms, nonseed plants and algae, with sub totals and totals in bold. The data used in TAPscan v1 (Lang et al. 2010) is compared with the present v2, divided into genomes and transcriptomes. Unpublished genomes and transcriptomes, which will be made available via the web interface upon publication, are listed.

To avoid sequences not encompassing the major part of the domain of interest, hit length and model usage had to be at least 75% of the model length, as mentioned above. For each of the 12 clades four sequences were sampled (if possible), before random sampling collected the remaining sequences to reach 50 sequences. If 50 sequences could not be sampled, due to too few hits in the 2010 (v1) output, all hits were used for building a new model. To measure the variability in the phylogenetically guided sampling approach it was repeated nine times. The detected domains from the chosen sampling run were then aligned using clustalw-2.1 (Larkin et al. 2007) and a new hmm3 model was built using hmmbuild. The new models were run against the same set of 46 genomes and the output scores were plotted (fig. 1*a*) and compared with the 2010 profile's findings (green in fig. 1*a*). To remain conservative, the sampling run that generated the profile that had the least amount of previously undetected sequences scoring higher than previously detected (diamond shaped in fig. 1*a*) was chosen for further processing. Defining the gathering cutoffs (ga_cut) of the profiles was done with the help of score-ordered multiple sequence alignments (fig. 1*b* and supplementary fig. S1, Supplementary Material online) visualized with Jalview v2.8.2 (Waterhouse et al. 2009). This made it possible to investigate each profiles' window of uncertainty with the aim to maintain physiochemical properties/conservation above the set ga_cut (cf. Results).

Updating Family Classification Rules

Using published detailed studies (see supplementary table S1, Supplementary Material online and Results for details) more subfamilies could be distinguished using both PFAM and novel custom profiles. By incorporating 9 new PFAM profiles and adding 5 new custom profiles, 11 additional TAP subfamilies could be added. This includes an expansion of the Homeodomain (HD) family from four to 12 subfamilies, an additional Jumonji subfamily, an additional Polycomb Group (PcG) subfamily, and being able to distinguish the MADS subclass MIKC. If no PFAM profile was available, custom profiles were made using existing multiple sequence alignments: BEL (Hamant and Pautot 2010; Sharma et al. 2014b), KNOX_C and PINTOX (Mukherjee et al. 2009) and WOX (van der Graaff et al.

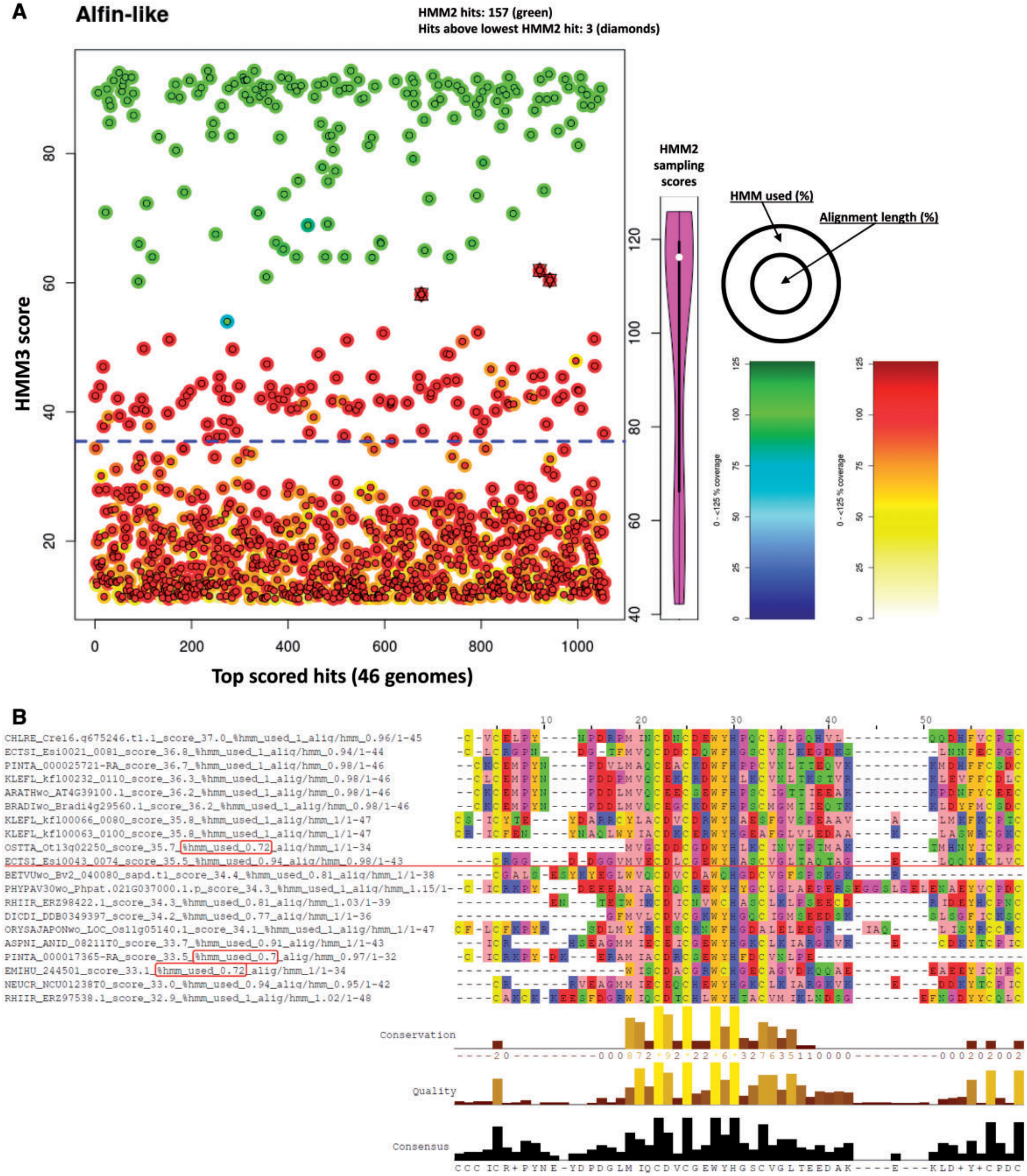


Fig. 1.—Determining gathering cutoffs for new custom profiles. (*A*) Plotted scores of the new profile (example: Alfin-like) run against 46 phylogenetically diverse genomes (supplementary table S2, Supplementary Material online). Sequences that were previously detected using the v1 profiles are colored in a green-blue gradient. New hits are colored in a red-yellow gradient. Each sequence's hit score is represented by an outer and inner area of the circle that represent the percentage hmm usage and alignment length, respectively. The dashed blue line represents the novel gathering cutoff, including sequences not previously captured (red circles above the line). The violin plot shows the old hmm2 score distribution of the sequences used to build the v1 model. If the new profile scored previously undetected sequences higher than previously detected sequences these are shown with diamond shapes. (*B*) A subsection of the sequence alignment of all hits (supplementary fig. S1, Supplementary Material online), highlighting where the gathering cutoff was set (red line, 34.5 in this example) based on manual inspection. The sequence names to the left of the alignment contain the five letter species code (supplementary table S2, Supplementary Material online) as well as the information of hmmsearch score and percentage of HMM used. Sequences later removed due to insufficient coverage (<75%) are marked with red boxes.

2009). When screening known PcG_EZ proteins (Pu and Sung 2015) the prosite CXC pattern (http://prosite.expasy.org/PS51633; last accessed December 8, 2017) was found and the underlying alignment used to build a custom model, replacing the SANTA domain (supplementary table S1, Supplementary Material online).

Inference of Ancestral States and Expansions/Contractions/Gains/Losses

We modified the ML phylogeny inferred by (Wickett et al. 2014) and placed our species into the clades included in their study. The tree was then pruned to only contain clades for which we had representative species (fig. 5). Our data included representatives of all major clades but hornworts, Magnoliids and Chloranthales, for which no appropriate data was available. This tree served as the basis for the inferences outlined below. Averages, fold changes between taxonomic groups and *q*-values (Mann–Whitney *U* test with Bonferroni correction for multiple testing) were calculated in Microsoft Excel (supplementary table S6, Supplementary Material online). Expansion/contractions and gains/losses were calculated with the count package (Csurös 2010). Their implementation of ancestral reconstruction by asymmetric Wagner parsimony was used to calculate expansions/contractions and their implementation of PGL (propensity for gene loss) was used to calculate gains/losses, both with default settings. All detected changes are shown in supplementary table S7, Supplementary Material online. The count predictions were entered into supplementary table S6, Supplementary Material online (tab Groups, column O-R) and manually reviewed; changes detected in (mainly) transcriptomic data/lineages with a low number of samples were disregarded, since they have a high chance of being due to incomplete data. Reviewed gains/losses/expansions/contractions were imposed onto the tree (fig. 5).

Phylogenetic Inference

The multiple sequence alignment of the DUF 632/PLZ family case study was calculated using muscle v3.8.31 (Edgar 2004) and visualized with Jalview v2.9.0b2. Sequences representing <50% of the alignment columns were removed and alignment columns with high entropy and low alignment quality as calculated by Jalview (Waterhouse et al. 2009) were manually clipped before Bayesian inference (BI) with MrBayes v3.2.5 x64 (Ronquist et al. 2012). The appropriate prior model was selected based on AIC/BIC using Prottest v3.4.2 (Darriba et al. 2011) and turned out to be JTT + G+F. BI was run with two hot and two cold chains until the standard deviation of split frequencies dropped < 0.01 at 756,600 generations, 200 trees were discarded as burn-in. The tree was visualized using FigTree v1.4.3pre (http://tree.bio.ed.ac.uk/software/figtree/; last accessed December 8, 2017).

For the gene trees shown in the TAPscan interface, we used several alignment tools as follows. Phylogenetic trees were generated for all TAPs appearing in more than one species of Archeaplastida. The protein sequences were downloaded using the TAPscan web interface and alignments were generated using MAFFT v7.310 (Katoh and Standley 2013). Alignments containing up to 500 input sequences were generated using MAFFT-linsi and MAFFT-fftnsi, whereas bigger alignments were generated only by MAFFT-fftnsi. The alignments were trimmed using two trimAl (Capella-Gutierrez et al. 2009) runs, one for trimming the alignments using the "-automated1" parameter and one for removing fragmentary sequences ("-resoverlap 0.75 -seqoverlap 50"). The trimmed mafft alignment was selected for inference if it was at least 100 columns long. If both linsi and fftnsi alignment were present and featured >100 columns, the longer one was selected.

If no suitable alignment could be generated, muscle v3.8.31 was run with two iterations and trimal applied. If that did not lead to a suitable trimmed alignment, ProbCons v1.12 (Do et al. 2005) was applied for alignments of up to 2, 100 input sequences. If that failed as well, muscle was applied with 16 iterations. In cases where trimAl produced empty/too short alignments, the automated trimming step was omitted. If all trimmed alignments were too short, the shortest untrimmed alignment was selected.

Alignments were formatted to Stockholm format using sreformat from the HMMer package. For neighbor-joining (NJ) tree inference, quicktree-SD (Frickenhaus and Beszteri 2008) was used applying using 100 bootstrap iterations. We used NJ inference due to the large to very large size of most of the gene families; in future trees generated with other methods of inference will be added. For visualization, the trees were formatted from Newick format to PhyloXML using the phyloxml (Han and Zmasek 2009) converter provided by the forester package (https://sites.google.com/site/cmzmasek/home/software/forester; last accessed December 8, 2017). The trees are presented on the TAPscan webpage using Archaeopteryx.js (https://sites.google.com/site/cmzmasek/home/software/archaeopteryx-js; last accessed December 8, 2017).

Visualization of Family Profiles and Column Charts

Using the R environment (R_Core_Team 2016) the family per species data (supplementary table S6, Supplementary Material online) was first log2 transformed and then hierarchically clustered on the *x* axis using complete linkage with euclidean distances to generate TAP clusters, and visualized as a heatmap using R gplots v3.0.1 (https://cran.r-project.org/web/packages/gplots/index.html; last accessed December 8, 2017). The *y* axis was ordered to follow our adaption of the (Wickett et al. 2014) phylogeny (fig. 5). The family per species data was also used to create stacked column charts (supplementary figs. S3 and S4, Supplementary Material online). Each TAP value was log2 transformed and then grouped by either TAP-class (supplementary fig. S3, Supplementary Material online) or amount of multiple domain TAPs (supplementary fig. S4, Supplementary Material online), maintaining the species separation.

Implementation of the TAPscan Online Resource

The web page was setup using a LAMP architecture (Lawton 2005) implemented with Linux Debian 9.1, Apache 2.4.25 (Debian), MariaDB 10.1.26 and PHP 7.0.19-1. PHP additionally uses HTML5, CSS3, Javascript and jQuery v3.1.1 for dynamic web page creation. The data used for the web page is saved as 18 tables which are normalized to avoid redundancy of the data. For example, there are five tables storing taxonomy information for the species table and two tables storing the domain rules for the domain and TAP family table. Access to the database is provided using PHP which also generates the HTML code sent to the user. The databases' entity relationship model is visualized in supplementary figure S7, Supplementary Material online. The gene trees and the underlying alignments (see above) were made available on the TAP family view pages for viewing and download.

Results and Discussion

Availability of accurate and state-of-the-art genome-wide TAP annotation is considered to be of high value, in particular for the plant science community. TAPscan v2 presents a framework for comparative studies of TAP function and evolution. The availability of new software tools, protein domain circumscriptions, and plant genomes triggered the updating of our previous rule sets and resources, and allowed to draw novel important conclusions on plant TAP evolution.

TAPscan v2 Uses More and Better Profiles

TAPscan relies on HMMs to detect domains. We updated our approach from using HMMER2 to its accelerated successor HMMER3 (http://hmmer.org/; last accessed December 8, 2017), making use of the novel local alignment of HMMs to define better coverage cutoffs. Moreover, we updated all used PFAM (Finn et al. 2016) profiles from version 23.0 to 29.0 and included nine new PFAM profiles (supplementary table S1, Supplementary Material online, columns "Additional Profiles" in the rule change tabs). Eight of those were added due to our novel diversified classification rules, and one previous custom profile, NAC_plant, was replaced with the now available PFAM profile NAM (supplementary table S1, Supplementary Material online). Among the updated PFAM HMMs, seven were renamed and two merged into other existing domain models. Out of nine name changes that occurred due to the PFAM updates, five affected domains of (previously) unknown function (supplementary table S1, Supplementary Material online, tab "name change").

We also added/exchanged five new custom-built profiles (BEL, KNOXC, PINTOX, WOX_HD, and CXC; cf. Methods) due to our expanded classification rules (supplementary table S1, Supplementary Material online, rule change tabs HD and PcG). All custom HMMs were updated using a phylogenetic sampling approach. For that, previously used HMMs (Lang et al. 2010) were run against a database of 46 genomes with broad phylogenetic sampling (supplementary table S2, Supplementary Material online). Using the 2010 (v1) profiles, hit sequences were sampled from each of the 12 groups that the 46 genomes represent, and then used to rebuild each custom HMM. The resulting HMMs were run against the same set of 46 genomes, and the outputs were compared to determine how previously undetected sequences scored now (fig. 1a). By manual inspection of all aligned hit sequences we defined the individual score cutoffs to lie above sequences of uncertain functional conservation (fig. 1*b* and supplementary fig. S1, Supplementary Material online). In order to represent a functionally relevant hit, the major part of the HMM should be detected. Based on manual inspection of all custom profile alignments we decided to employ a global cutoff of 75% HMM used (fig. 1*b* and supplementary fig. S1, Supplementary Material online).

Improved Taxon Sampling, Subfamily Definition, and Specificity

In the past 7 years, a multitude of plant and algal genome sequences became available, allowing for a much better taxon sampling. There are now 82 more plant genomes included in TAPscan v2, and nine more algal genomes, bringing the total up to 110 (table 1). To improve taxonomic resolution we also included a selection of 13 transcriptomes, reaching a final set of 123 species. We have also included 11 genomes and 1 transcriptomes that are not yet published. Data for those will be quickly made available via the web interface as soon as they are publicly available. For example, PlantTFDB v4 (Jin et al. 2016) includes more angiosperm genomes, we took care to include as much as possible nonseed plants and streptophyte algae, to be able to take a close look at the early evolution of plant TAPs. In addition to the Viridiplantae that are the focus of this study, we have included Rhodophyta and the glaucophyte alga *Cyanophora paradoxa* as outgroup representatives within the Archaeplastida (supplementary table S5/S6, Supplementary Material online).

To update our classification rules (supplementary table S3, Supplementary Material online), we screened the literature for novel (sub) classifications of TAPs and checked them for applicability to our domain-based classification scheme. In total, 11 new subfamily classification rules were established, and some families renamed due to changes in domain or family names (fig. 2). In particular, we subdivided homeodomain

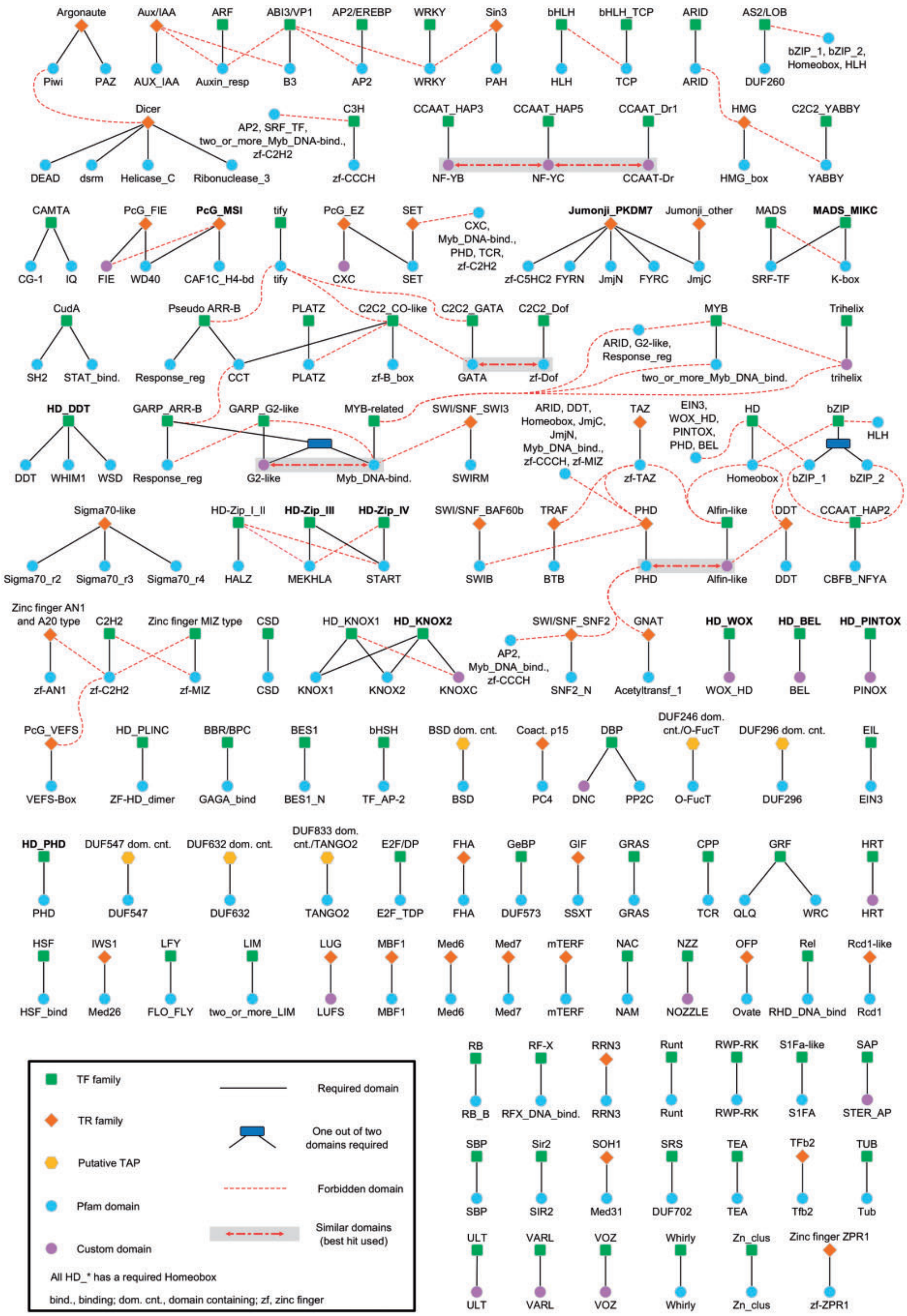


FIG. 2.—TAPscan classification rules. The name of each family or subfamily is shown on top of each classification rule set; novel (in v2) rule sets are shown in bold face. TF (green), TR (orange), and PT (yellow) are marked by different symbols and in the same color code that is used throughout the manuscript. Required ("should") domains (represented by corresponding HMMs) are connected to the family symbol by lines; forbidden ("should not") domains are connected via dotted red lines. Similar domains that are selected via the best hit are shown with red dotted double arrows on grey background, if one out of two domains are required this is denoted as a blue box with two required lines. Custom domains are depicted as purple circles, PFAM domains as blue circles. For brevity, the homeobox should rule for all HD_families was omitted. Compare supplementary table S3, Supplementary Material online for more detailed classification rules.

(HD) TFs into DDT, PHD, PINTOX, PLINC, WOX, HD-ZIP I/II, III, IV, and into the TALE class subfamilies BEL, KNOX 1, and 2 (Mukherjee et al. 2009; van der Graaff et al. 2009; Hamant and Pautot 2010; Sharma et al. 2014b) (supplementary table S1, Supplementary Material online, 1st sheet). Also, MADS-box TFs were divided into general and MIKC-type (Gramzow and Theissen 2010), Jumonji into PKDM7 and other (Qian et al. 2015), and the Polycomb Group (PcG) TR MSI was added (supplementary table S1, Supplementary Material online). Similar to (Zheng et al. 2016) we reclassified mTERF, Sigma70-like, FHA and TAZ as TR instead of TF; TAPs containing the DDT domain are subdivided into the TR DDT and the TF HD_DDT in TAPscan v2. With a total of 124 families and subfamilies (supplementary table S3, Supplementary Material online, fig. 2; 81 of them TFs) our rule set is the most comprehensive one for plant TAPs, since other approaches have significantly less resolution, for example, 58 in PlantTFDB 4.0 (Jin et al. 2016) and 72 in iTAK (Zheng et al. 2016).

We compared the TAPscan v1 and v2 annotations with a number of *A. thaliana* and *P. patens* phylogeny-based family classifications defined as gold standard (Mosquna et al. 2009; Mukherjee et al. 2009; Paponov et al. 2009; Martin-Trillo and Cubas 2010). We find that the average sensitivity of TAPscan v2 (87.76%) is only slightly lower than of v1 (89.31%), whereas the specificity of v2 (100.00%) is much higher than in the old version (92.31%; supplementary table S4, Supplementary Material online). The combined sensitivity and specificity of the new version is therefore 6.1% improved. It should be noted that the comparatively low sensitivity for some of the HD sub classes is balanced by the fact that all HD family members are detected as such, yet in cases where domain scores are below cutoffs are sometimes binned into HD_other. The weighted sensitivity, taking into account gene family sizes, is strongly improved to 87.03% as compared with 78.27% in (Lang et al. 2010).

The TAPscan Online Resource

In order to make the domain-based classification available to the scientific community in an easy to use way, we implemented a web-based resource that allows a user to browse the data either in a species-centric or a TAP family-centric view (http://plantco.de/tapscan/). The interface (fig. 3) includes taxonomic information as expandable trees and an intuitive click-system for selection of sequences of interest that can subsequently be downloaded in annotated FASTA format. TAPscan FASTA headers contain the species, TAP family information and domain positions. It is possible to either download all proteins of a custom set of species containing a specific TAP, or to download all proteins for a specific family and species. The latter makes it possible to download isoforms, if available.

The TAP overview pages show the domain rules a protein has to meet in order to be classified as belonging to that family. Domain names are linked to PFAM entries or custom domain alignments and HMM profiles. Locations of domains within the sequence are shown in sequence view. Precomputed phylogenies (gene trees) are available for viewing and download on the overview pages. These trees are intended as a first glimpse, allowing users to quickly access gene relationships without having to infer a tree on their own.

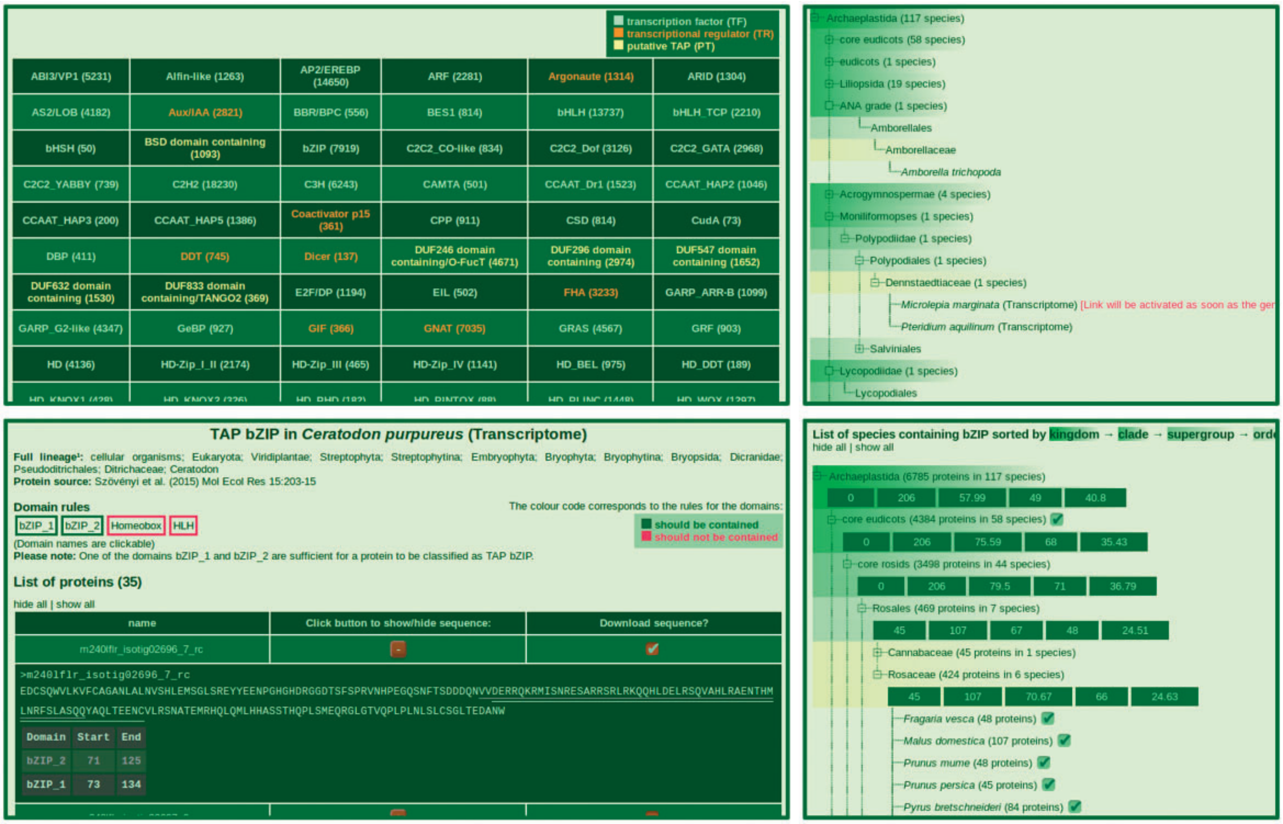
In the case of not yet published sequence data (table 1) a disclaimer is shown, mentioning that the data will be made available immediately upon publication. Such unpublished information is excluded from species or protein counts in the web interface. By including these data into the interface we are able to quickly release TAP annotation for these genomes as soon as the data become public.

Taxonomic Profiling of TAPs

Heatmap representation of the data shows that TAP family size generally increased during land plant evolution (fig. 4 and see supplementary fig. S2, Supplementary Material online for expanded version). Cluster 5 contains families (such as bZIP, bHLH, or MYB) that were already abundant in the algal relatives of land plants, whereas cluster 3 contains TAPs that expanded in land plants and again in seed plants, such as NAC or ABI3/VP1. The intervening cluster 4 contains families that show high abundance throughout, like HD or RWP-RK. The biggest cluster (1) contains families that show either only gradual expansion from algae (bottom of figure) to flowering plants (top of figure), or no expansion at all. Consequently, cluster 1 contains many TRs, which have previously shown not to be subject to as much expansion as TFs (Lang et al. 2010). The small cluster 2 next to cluster 1 harbors families of spurious presence, like those that evolved in vascular plants (Tracheophyta; like NZZ or ULT). In general, the heatmap visualizes a principal gain of (primarily) TF paralogs within existing families concomitant with the terrestrialization of plants (cf. supplementary fig. S3, Supplementary Material online). Interestingly, the propensity of TAPs to comprise of more than one functional domain increases in a very similar pattern (supplementary fig. S4, Supplementary Material online), akin to the domain combination tendency generally seen for plants (Kersting et al. 2012). Hence, the combinatorial potential of TFs clearly coincides with increasing morphological complexity (as measured by number of cell types), corroborating earlier results (Lang et al. 2010; Lang and Rensing 2015).

TAP Family Evolution

The taxonomic sampling of our data is visualized as a cartoon tree (fig. 5) derived from a recent phylogenomics study (Wickett et al. 2014). We plotted the gains, losses, expansions and contractions of TAP families onto this tree to enable a global view of plant TAP evolution (cf. supplementary table



🟩 transcription factor (TF)
🟧 transcriptional regulator (TR)
🟨 putative TAP (PT)

ABI3/VP1 (5231)	Alfin-like (1263)	AP2/EREBP (14650)	ARF (2281)	Argonaute (1314)	ARID (1304)
AS2/LOB (4182)	Aux/IAA (2821)	BBR/BPC (556)	BES1 (814)	bHLH (13737)	bHLH_TCP (2210)
bHSH (50)	BSD domain containing (1093)	bZIP (7919)	C2C2_CO-like (834)	C2C2_Dof (3126)	C2C2_GATA (2968)
C2C2_YABBY (739)	C2H2 (18230)	C3H (6243)	CAMTA (501)	CCAAT_Dr1 (1523)	CCAAT_HAP2 (1046)
CCAAT_HAP3 (200)	CCAAT_HAP5 (1386)	Coactivator p15 (361)	CPP (911)	CSD (814)	CudA (73)
DBP (411)	DDT (745)	Dicer (137)	DUF246 domain containing/O-FucT (4671)	DUF296 domain containing (2974)	DUF547 domain containing (1652)
DUF632 domain containing (1530)	DUF833 domain containing/TANGO2 (369)	E2F/DP (1194)	EIL (502)	FHA (3233)	GARP_ARR-B (1099)
GARP_G2-like (4347)	GeBP (927)	GIF (366)	GNAT (7035)	GRAS (4567)	GRF (903)
HD (4136)	HD-Zip_I_II (2174)	HD-Zip_III (465)	HD-Zip_IV (1141)	HD_BEL (975)	HD_DDT (189)
HD_KNOX1 (428)	HD_KNOX2 (326)	HD_PHD (182)	HD_PINTOX (88)	HD_PLINC (1448)	HD_WOX (1297)

```
⊟-Archaeplastida (117 species)
  ⊞-core eudicots (58 species)
  ⊞-eudicots (1 species)
  ⊞-Liliopsida (19 species)
  ⊟-ANA grade (1 species)
      └-Amborellales
          └-Amborellaceae
              └-Amborella trichopoda
  ⊞-Acrogymnospermae (4 species)
  ⊟-Moniliformopses (1 species)
      ⊟-Polypodiidae (1 species)
          ⊟-Polypodiales (1 species)
              ⊟-Dennstaedtiaceae (1 species)
                  ├-Microlepia marginata (Transcriptome) [Link will be activated as soon as the ger
                  └-Pteridium aquilinum (Transcriptome)
          ⊞-Salviniales
  ⊟-Lycopodiidae (1 species)
      └-Lycopodiales
```

TAP bZIP in *Ceratodon purpureus* (Transcriptome)

Full lineage¹: cellular organisms; Eukaryota; Viridiplantae; Streptophyta; Streptophytina; Embryophyta; Bryophyta; Bryophytina; Bryopsida; Dicranidae; Pseudoditrichales; Ditrichaceae; Ceratodon
Protein source: Szövényi et al. (2015) Mol Ecol Res 15:203-15

Domain rules

bZIP_1 bZIP_2 Homeobox HLH
(Domain names are clickable)
Please note: One of the domains bZIP_1 and bZIP_2 are sufficient for a protein to be classified as TAP bZIP.

The colour code corresponds to the rules for the domains:
🟩 should be contained
🟥 should not be contained

List of proteins (35)

hide all | show all

name	Click button to show/hide sequence:	Download sequence?
m240lflr_isotig02696_7_rc	-	☑

```
>m240lflr_isotig02696_7_rc
EDCSQWVLKVFCAGANLALNVSHLEMSGLSREYYEENPGHGHDRGGDTSFSPRVNHPEGQSNFTSDDDQNVVDERRQKRMISNRESARRSRLRKQQHLDELRSQVAHLRAENTHM
LNRFSLASQQYAQLTEENCVLRSNATEMRHQLQMLHHASSTHQPLSMEQRGLGTVQPLPLNLSLCSGLTEDANW
```

Domain	Start	End
bZIP_2	71	125
bZIP_1	73	134

List of species containing bZIP sorted by kingdom → clade → supergroup → ord
hide all | show all

```
⊟-Archaeplastida (6785 proteins in 117 species)
   0 | 206 | 57.99 | 49 | 40.8
  ⊟-core eudicots (4384 proteins in 58 species) ☑
     0 | 206 | 75.59 | 68 | 35.43
    ⊟-core rosids (3498 proteins in 44 species)
       0 | 206 | 79.5 | 71 | 36.79
      ⊟-Rosales (469 proteins in 7 species)
         45 | 107 | 67 | 48 | 24.51
        ⊞-Cannabaceae (45 proteins in 1 species)
        ⊟-Rosaceae (424 proteins in 6 species)
           45 | 107 | 70.67 | 66 | 24.63
            ├-Fragaria vesca (48 proteins) ☑
            ├-Malus domestica (107 proteins) ☑
            ├-Prunus mume (48 proteins) ☑
            ├-Prunus persica (45 proteins) ☑
            └-Pyrus bretschneideri (84 proteins) ☑
```

Fig. 3.—TAPscan web interface main features. Upper left: Family-centric view—table of TAP families covered by TAPscan; the number of proteins per family is given in brackets. TAPs are colored according to their TAP class (TF, TR, and PT). Upper right: Species-centric view—part of the species tree; different levels can be expanded and collapsed. Numbers of published species per taxonomy level are given in brackets. Only species with published protein data can be accessed. Bottom left: Species view for TAP family bZIP in *Ceratodon purpureus*. The species' lineage, the bZIP domain rules, and the protein sequences are shown. One protein is marked for downloading. Bottom right: Species tree for the bZIP family with expanded SAR kingdom. Species belonging to Alveolata are marked for downloading; the resulting file will contain 54 proteins. TAP distribution is given in a table-like manner, with a dark green background: minimum, maximum, average, median, and standard deviation of proteins per species for the selected taxonomy level.

S6/S7, Supplementary Material online). 32 losses were predicted that are scattered along the tree. The streptophyte alga *Klebsormidium nitens* apparently secondarily lost five TAP families, whereas the lycophyte *Selaginella moellendorffii* lost eight. Another eight families were lost during gymnosperm evolution, one of them (HD_Pintox) being absent from all studied gymnosperms, whereas two are lacking in conifers and five in *Ginkgo* (e.g., LFY—although a lacking gene model would be an alternative explanation). A total of 76 expansions were detected, of which the highest number (17.22%) are inferred to have occurred in the lineage that led to the last common ancestor of all land plants. All other expansions show a scattered distribution along the deep as well as distal nodes of the tree (fig. 5). The 13 inferred family contractions also display a patchy pattern. Strikingly, out of 36 TAP family gains 26 are predicted to have occurred in streptophyte algae (nodes 34-30). Another five are synapomorphic of land plants (Embryophyta), whereas only 2, 1, and 1 are evolutionary novelties of vascular plants, Euphyllophyta, and Eudicots, respectively.

Many TAP Families Were Gained in the Water

Previously, due to limited taxon sampling, many plant-specific TAPs were inferred to have been gained at the time of the water-to-land-transition of plant life (Lang et al. 2010). Streptophyte algae are sister to land plants and thus ideally suited, together with bryophyte sequences, to elucidate whether gains occurred prior or after terrestrialization. Although only two genomes of strepptophyte algae have yet been published (Hori et al. 2014; Delaux et al. 2015), there are transcriptome data available for seven species (Timme et al. 2012) that were included into TAPscan (table 1 and supplementary table S5/S6, Supplementary Material online). Similarly, although no other bryophyte genomes than *P. patens* are published yet, we included transcriptomes of the mosses *Ceratodon purpureus* (Szovenyi et al. 2015) and *Funaria hygrometrica* (Szovenyi et al. 2011), and of the liverwort *Marchantia polymopha* (Sharma et al. 2014a). Out of 20 TAP families previously thought to have been gained with terrestrialization (Lang et al. 2010), only VOZ and bHLH_TCP

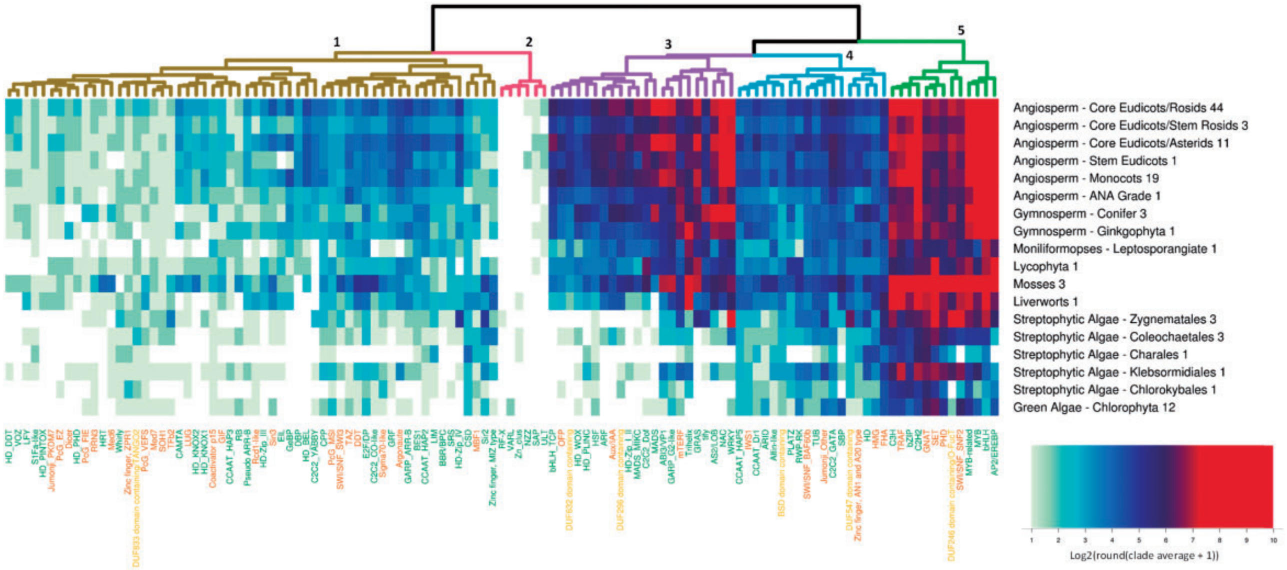


Fig. 4.—TAPfamily abundance heat map. Heatmap using log2 transformed average values of TAP abundance for each clade. The data was clustered on the *x* axis using complete linkage with euclidean distances. The *y* axis was kept to match the phylogeny as in Wickett et al. (2014), cf. figure 5. The logarithmic color scheme comprises white (absent) through blue to red (high abundance).

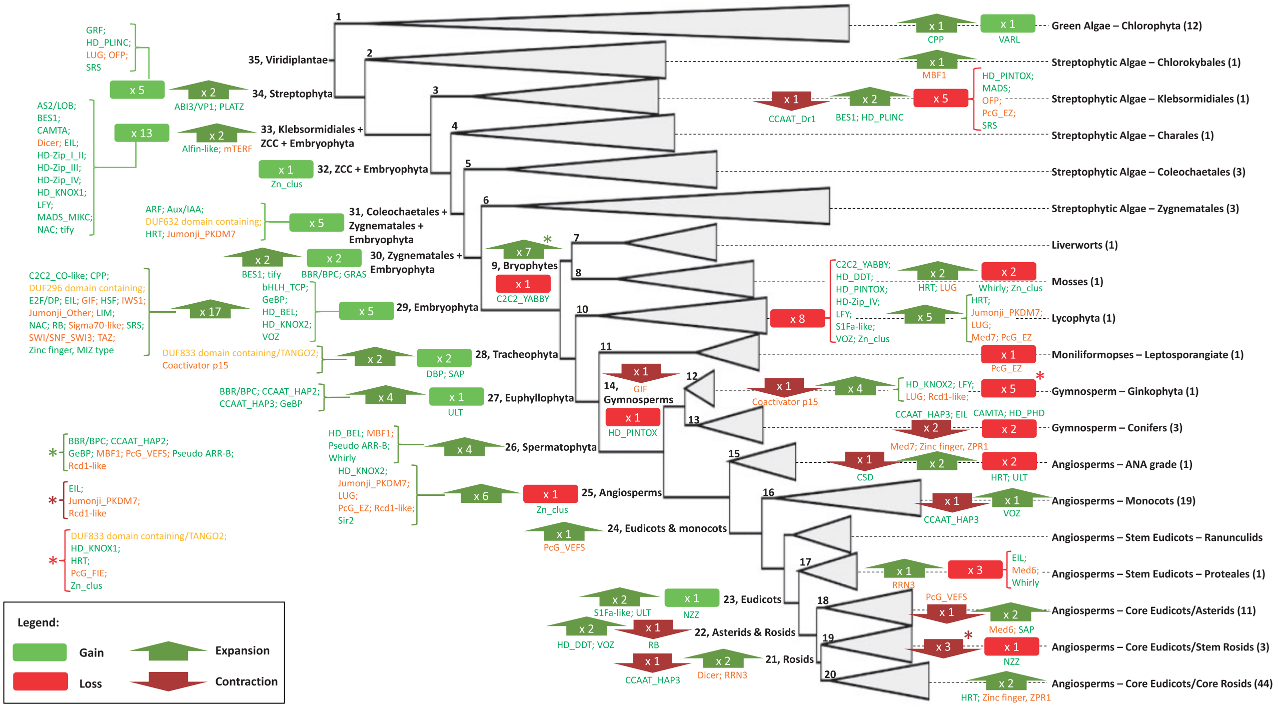

1
2
3
4
5
6
7
8
10
11
12
13
15
16
17
18
19
20
35, Viridiplantae
34, Streptophyta
33, Klebsormidiales + ZCC + Embryophyta
32, ZCC + Embryophyta
31, Coleochaetales + Zygnematales + Embryophyta
30, Zygnematales + Embryophyta
9, Bryophytes
29, Embryophyta
28, Tracheophyta
27, Euphyllophyta
14, Gymnosperms
26, Spermatophyta
25, Angiosperms
24, Eudicots & monocots
23, Eudicots
22, Asterids & Rosids
21, Rosids
GRF; HD_PLINC; LUG; OFP; SRS
x 5
x 2
ABI3/VP1; PLATZ
AS2/LOB; BES1; CAMTA; Dicer; EIL; HD-Zip_I_II; HD-Zip_III; HD-Zip_IV; HD_KNOX1; LFY; MADS_MIKC; NAC; tify
x 13
x 2
Alfin-like; mTERF
x 1
Zn_clus
ARF; Aux/IAA; DUF632 domain containing; HRT; Jumonji_PKDM7
x 5
x 2
BES1; tify
x 2
BBR/BPC; GRAS
x 7
*
x 1
C2C2_YABBY
C2C2_CO-like; CPP; DUF296 domain containing; E2F/DP; EIL; GIF; HSF; IWS1; Jumonji_Other; LIM; NAC; RB; Sigma70-like; SRS; SWI/SNF_SWI3; TAZ; Zinc finger, MIZ type
x 17
bHLH_TCP; GeBP; HD_BEL; HD_KNOX2; VOZ
x 5
DUF833 domain containing/TANGO2; Coactivator p15
x 2
x 2
DBP; SAP
BBR/BPC; CCAAT_HAP2; CCAAT_HAP3; GeBP
x 4
x 1
ULT
x 1
GIF
x 1
HD_PINTOX
HD_BEL; MBF1; Pseudo ARR-B; Whirly
x 4
HD_KNOX2; Jumonji_PKDM7; LUG; PcG_EZ; Rcd1-like; Sir2
x 6
x 1
Zn_clus
x 1
PcG_VEFS
x 2
S1Fa-like; ULT
x 1
NZZ
x 2
HD_DDT; VOZ
x 1
RB
x 1
CCAAT_HAP3
x 2
Dicer; RRN3
* BBR/BPC; CCAAT_HAP2; GeBP; MBF1; PcG_VEFS; Pseudo ARR-B; Rcd1-like
* EIL; Jumonji_PKDM7; Rcd1-like
* DUF833 domain containing/TANGO2; HD_KNOX1; HRT; PcG_FIE; Zn_clus
Legend:
Gain
Loss
Expansion
Contraction
x 1
CPP
x 1
VARL
Green Algae – Chlorophyta (12)
x 1
MBF1
Streptophytic Algae – Chlorokybales (1)
x 1
CCAAT_Dr1
x 2
BES1; HD_PLINC
x 5
HD_PINTOX; MADS; OFP; PcG_EZ; SRS
Streptophytic Algae – Klebsormidiales (1)
Streptophytic Algae – Charales (1)
Streptophytic Algae – Coleochaetales (3)
Streptophytic Algae – Zygnematales (3)
Liverworts (1)
C2C2_YABBY; HD_DDT; HD_PINTOX; HD-Zip_IV; LFY; S1Fa-like; VOZ; Zn_clus
x 8
x 2
HRT; LUG
x 2
Whirly; Zn_clus
Mosses (1)
x 5
HRT; Jumonji_PKDM7; LUG; Med7; PcG_EZ
Lycophyta (1)
x 1
PcG_EZ
Moniliformopses – Leptosporangiate (1)
x 1
Coactivator p15
x 4
HD_KNOX2; LFY; LUG; Rcd1-like;
x 5
*
Gymnosperm – Ginkophyta (1)
CCAAT_HAP3; EIL
x 2
Med7; Zinc finger, ZPR1
CAMTA; HD_PHD
x 2
Gymnosperm – Conifers (3)
x 1
CSD
x 2
x 2
HRT; ULT
Angiosperms – ANA grade (1)
x 1
CCAAT_HAP3
x 1
VOZ
Angiosperms – Monocots (19)
Angiosperms – Stem Eudicots – Ranunculids
x 1
RRN3
x 3
EIL; Med6; Whirly
Angiosperms – Stem Eudicots – Proteales (1)
PcG_VEFS
x 1
x 2
Med6; SAP
Angiosperms – Core Eudicots/Asterids (11)
*
x 3
x 1
NZZ
Angiosperms – Core Eudicots/Stem Rosids (3)
x 2
HRT; Zinc finger, ZPR1
Angiosperms – Core Eudicots/Core Rosids (44)

Fig. 5.—Cartoon tree illustrating the predicted ancestral states, expansion/contractions, and gains/losses of plant TAPs. The tree was modified from Wickett et al. (2014); number of data sets covered per clade is shown in brackets. Gains and losses were predicted using PGL, expansions and contractions using Wagner parsimony (cf. Methods and supplementary table S7, Supplementary Material online). These predictions were entered into supplementary table S6, Supplementary Material online (tab Groups, column O-R) and manually reviewed; changes detected in (mainly) transcriptomic data/lineages with a low number of samples were disregarded, since they have a high chance of being due to incomplete data. Reviewed gains/losses/expansions/contractions of TFs (green text), TRs (orange text), and PTs (yellow text) were imposed onto the tree: gains are shown as green boxes, losses as red boxes. Expansions are shown as green upward arrows, contractions as red downward arrows. Node numbers and names are as in supplementary table S7, Supplementary Material online; symbols are shown to the right of triangles if they concern a distal node, and to the left if they concern a deep node.

could be confirmed. Of the others, three (ARF, S1Fa-like, and O-FucT) are already present in Rhodophyta, Chlorophyta, or both. Strikingly, the vast majority of these 20 families (15) are present in Charophyta (comprising all lineages of streptophyte algae), but not Chlorophyta or Rhodophyta (supplementary table S6, Supplementary Material online). Hence, they were most probably gained during the evolution of the Streptophyta (uniting the Charophyta with the land plants). Out of these 15 families, 11 are already present in the KCM grade (encompassing Klebsormidiales, Chlorokybales, and Mesostigmatales and sister to the ZCC grade and land plants), whereas 4 (Aux/IAA, DUF632 domain containing, GRAS and HRT) are present only in the ZCC grade (encompassing Zygnematales, Coleochaetales, and Charales, together with the land plants comprising the Phragmoplastophyta). This finding is in line with the emerging evidence that in particular ZCC species share many unique features with land plants like polyplastidy (de Vries et al. 2016) or the phragmoplast (Pickett-Heaps et al. 1999; Buschmann and Zachgo 2016), and that Klebsormidium also possesses some "plant-like" features, like callose and the phenylpropanoid pathway (Herburger and Holzinger 2015; de Vries et al. 2017). Based on our findings, the last common ancestor of streptophytes had already evolved 11 TAP families previously thought to be land plant-specific, and the last common ancestor of Phragmoplastophyta (ZCC grade algae and land plants) another five families. Prominent examples of these families are the TF families LFY and NAC (present already in *K. nitens*), as well as GRAS and Aux/IAA (present in the ZCC grade). Most of what we know about function of these TF families stems from research in flowering plants, and many of them control development of organs unique to flowering plants. It will therefore be intriguing to determine the putative ancestral function of these genes in the last common ancestor of streptophytes. As an example, a recent study showed that a *P. patens* TCP TF is involved in suppressing branching of the moss sporophyte (which is determinate since it does not branch) (Ortiz-Ramirez et al. 2016).

Origin and Expansion Revisited

Several of the gains previously inferred to have occurred in vascular plants, angiosperms or eudicotelydons can now be dated back to the common ancestors with streptophyte algae, bryophytes, ferns or lycophytes (fig. 5 and supplementary table S6/S7, Supplementary Material online). Together with the families mentioned in the last paragraph, a total of 35 TAP families (most of them TFs) evolved at some point in the Archaeplastida, before the evolution of angiosperms, shifting the inferred gain dates back in time. Yet, out of 44 TAP families previously inferred to be expanded in land plants as compared with algae (Lang et al. 2010), 21 show a >2-fold increase in the data presented here, and all 44 significantly more members ($q < 0.05$, Mann–Whitney) in land plants than in algae (supplementary table S6, Supplementary Material online). These data suggest a primary burst of gain and expansion of TAPs concomitant with the origin of Streptophyta. The total numbers of TAPs, and in particular TFs, show a clear increase in the common ancestor of land plants, but also in some streptophyte algae (supplementary fig. S3, Supplementary Material online). We expect that with more genomes of streptophyte algae becoming available the gain and expansion of even more families will be inferred to have occurred at earlier time points.

Of 22 families previously inferred to have been expanded in angiosperms (Lang et al. 2010), the present data support 17 with a 2-fold change and 15 based on statistical testing (overlap 13; $q < 0.05$; supplementary table S6, Supplementary Material online). Six TAP families expanded at the basis of angiosperms (among them HD_KNOX2), and several families expanded subsequently (fig. 5). The subfunctionalization of such TAPs might be related to the more complex reproductive system of angiosperms. While most TF were already present in the earliest land plants, DBP and SAP appear first in vascular plants, ULT in the common ancestor of ferns and seed plants, and NZZ is unique to eudicots.

One of the major gaps in the previous sampling, besides the streptophyte algae, were gymnosperms. We have now included three conifers and *Ginkgo biloba*. If we consider the inferred expansions based on the tree (fig. 5), a total of 13 expansions occur between the land plant node (29) and the angiosperms (25). Four TF families (BBR/BPC, CCAAT_HAP2, CCAAT_HAP3, and GeBP) were apparently expanded in the Euphyllophyta (ferns and seed plants, node 27), another three (HD_BEL, Pseudo ARR-B, and Whirly) in the seed plants. All these TF families are thus presumably important for spermatophyte evolution and development.

In a recent study (Catarino et al. 2016), the authors had analyzed 48 plant TF families based on PlantTFDB classification rules (Jin et al. 2014) in 15 species. In general, their inference of TF family gain is consistent with our data: of 38 families that can be compared, 30 are placed at the same node. For the remaining eight, our study places six at earlier nodes of the tree, probably due to better taxon sampling. The study also did a subfamily analysis of HD TFs and concluded that almost all were already present in algae. In our study, we find that of 12 HD subfamilies all but two (HD_BEL and HD_KNOX2) are detected in algae. We also compared gain of 40 TF families from (Jin et al. 2016) with our data and can confirm their findings for 27 families. Out of the remaining 13, we detect 10 at earlier nodes in the tree, 4 of them in ZCC grade streptophyte algae instead of bryophytes, suggesting again that due to better sampling we infer family evolution more accurately. The *M. polymorpha* genome was published (Bowman et al. 2017) during the time this manuscript was under review. We have hence activated the previously computed data in the web interface and have added corresponding columns to supplementary table S6, Supplementary Material online; the comparison of the transcriptomic and genomic data does not show any severe differences. The genome publication included an analysis of TFs that we compared with our data (supplementary table S6, Supplementary Material online). We detect 400 TFs, Bowman et al. 387 TFs; 33 out of 40 families are consistent; in the remaining seven cases the node of predicted origin varies due to different sampling.

Employing TAPscan Data

As an example on how the data presented with this study can be used, we selected the putative TAP family "DUF 632 domain containing." This domain of unknown function (http://pfam.xfam.org/family/PF04782; last accessed December 8, 2017) is described as representing a potential leucine zipper, which is why it was initially defined as a putative TAP, PT (Richardt et al. 2007). Our data show that this family first appears in the common ancestor of Coleochaetales, Zygnematales, and land plants (node 31) and is present throughout land plants (supplementary table S6, Supplementary Material online, and fig. 5). There are on average 19 family members in angiosperms, 7 in gymnosperms, 6 in bryophytes, and 3 in the streptophyte alga *Coleochaete orbicularis*. DUF 632 is part of cluster 3 (fig. 4) that shows expansion during land plant evolution. It is not detected to be expanded using Wagner parsimony (fig. 5), but shows significant size increase ($q < 0.05$, Mann–Whitney; supplementary table S6, Supplementary Material online) between nonseed plants and seed plants (fold change 2.95).

We selected protein sequences of this family using the TAPscan interface "family view" option, thus representing several angiosperm lineages as well as gymnosperms and nonseed plants. An alignment of the sequences (supplementary fig. S5, Supplementary Material online) shows several highly conserved blocks, all of which feature positively charged as well as regularly spaced Leucine residues, reinforcing the notion of a potential DNA-binding Leucine zipper. Given the proposed structure we suggest to call this family Plant Leucine Zipper (PLZ) TFs. Phylogenetic inference shows that all nonseed plant sequences are present in the same subclade (supplementary fig. S6, Supplementary Material online; the same can be derived from the tree automatically inferred and available via the TAPscan web interface), this subclade is sister to approximately half of the seed plant sequences. Based on the structure of the tree, duplication and paralog retention occurred several times during seed plant evolution. Most of the paralogs were already established in the lineage leading to the last common ancestor of seed plants, whereas some duplications occurred only in angiosperms.

In order to understand under which conditions members of this protein family are active, we conducted expression profiling using existing data for *P. patens* and *A. thaliana* (Hruz et al. 2008; Hiss et al. 2014, 2017, phytozome.org). Out of five *P. patens* genes detected by TAPscan, one appears to be a truncated pseudogene that was removed during alignment curation; another two genes are barely expressed. The remaining two genes (Pp3c16_15000V3.1 and Pp3c27_2840V3.1), however, show discrete expression profiles. The expression of both genes is higher under diurnal light and ammonia application. Pp3c16_15000V3.1 is more highly expressed upon heat stress, darkness and UV-B treatment, as well as in mature sporophytes and under biotic stimulus. Pp3c27_2840V3.1 is less expressed in gametophores (representing the late vegetative phase) as well as in mature sporophytes (i.e., adversely to the other gene). Similarly, ABA treatment leads to lower expression of Pp3c16_15000V3.1 and higher expression of Pp3c27_2840V3.1. The two *A. thaliana* genes most closely related to the nonseed plant clade, AT5G25590.1 and AT1G52320.2, show no particularly strong expression in any tissue or developmental stage, however, other members of the family show peaks in, for example, reproductive structures, xylem, or seed. AT1G52320.2 is induced, for example, under germination, drought and ABA, whereas AT5G25590.1 shows higher expression, for example, under UV-B, biotic stimulus, elevated CO_2 and drought. In summary, members of the streptophyte-specific PLZ family appear to be differentially regulated under a range of abiotic and biotic stimuli as well as in different development stages. Such an expression profile fits that of a TF family undergoing paralog retention followed by sub and neofunctionalization of expression domains (Birchler and Veitia 2010; Rensing 2014).

Outlook

Previous studies of land plant TAP evolution, like (Lang et al. 2010), suffered from severe sampling bias, leading to many gains and expansions being either associated with the water to land transition (because they were inferred to have occurred between green algae and the moss *P. patens*), or the angiosperm radiation (since they occurred between the lycopyhte *S. moellendorffii* and angiosperms). Using better sampling, including streptophyte algae, more bryophytes, a fern and gymnosperms, we can now more accurately trace Viridiplantae TAP gains and expansions. Although we expect that we will have to again adjust our current understanding as more genomes become available, we can now say that much of what we considered to be specific for land plants or flowering plants already evolved in the water, in streptophyte algae, or in the course of preflowering land plant evolution.

The results of our improved genome-wide TAP annotation methodology, including annotated fasta files and gene trees, are now available online via an easy-to-use web interface. Species already sequenced but not yet published have already been included and will be made available immediately after publication. We trust that TAPscan v2 will be an important community resource for plant TAP analyses.

Supplementary Material

Supplementary data are available at *Genome Biology and Evolution* online.

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Author Contributions

S.A.R. conceived of the study, supervised it, wrote the paper and carried out evolutionary and phylogenetic analyses. K.K.U. was in charge of setting up the genomic data. P.K.I.W. adapted the TAPscan tool, carried out TAP classification and analyzed data. P.K.I.W. and K.K.U. implemented the phylogenetic sampling. C.M., K.K.U., and S.A.R. inferred gene trees. C.M. established the web interface. All authors contributed to writing the manuscript.

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