COUNTING TREE-CHILD NETWORKS AND THEIR SUBCLASSES

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Abstract. Galled trees are studied as a recombination model in population genetics. This class of phylogenetic networks is generalized into tree-child, galled and reticulation-visible network classes by relaxing a structural condition imposed on galled trees. We count tree-child networks through enumerating their component graphs. Explicit counting formulas are also given for galled trees through their relationship to ordered trees, phylogenetic networks with few reticulations and phylogenetic networks in which the child of each reticulation is a leaf.

Key words. Galled networks, normal networks, tree-child networks, tree-based networks

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1. Introduction. Phylogenetic networks have been used often to model gene and genome evolution over recent years [14, 20]. A rooted phylogenetic network (RPN) is a rooted directed acyclic graph (DAG) where all the sink nodes are of indegree 1, called the leaves, and where there is a unique source node, called the root.

The leaves represent a set of taxa (e.g., species, genes or individuals in a population) and the root represents the least common ancestor of the taxa. Moreover, in a RPN, non-leaf and non-root nodes are divided into two classes: tree nodes, which have more children than parents, and reticulations, which have more parents than children.

It is a great challenge to reconstruct phylogenetic networks from sequence or gene tree data [18]. Imposing topological conditions on networks allows us to define simple classes of RPNs, on which evolution can hopefully be understood well [14, 16, 21]. One network class is tree-child networks (TCNs) [4], in which every non-leaf node has a child that is a tree node or a leaf. Other popular network classes include galled trees [15, 27], normal networks [28], galled networks [17], reticulation-visible networks [19] and tree-based networks [7, 29] (see also [26, 30]). Indeed, the tree and cluster containment problems that are NP-complete in general become solvable in polynomial time when restricted to all but tree-based network class [1, 9, 10, 11].

In this paper, we investigate how to count TCNs and other related classes. Phylogenetic trees are RPNs with no reticulations. It is known that there are $2^{1-n}(2n-2)!/(n-1)!$ binary phylogenetic trees on $n$ taxa. However, counting becomes much harder for RPNs [8, 22]. Recently, progresses have been made for TCNs, normal networks and galled trees. Semple and Steel first studied how to count unrooted galled trees. They gave closed formulas for the number of unrooted galled trees in terms of two parameters: the number of galls and the total number of edges over all the gall cycles [24]. Bouvel et al. presented generating functions and explicit formulas for count of unrooted and rooted galled trees, as well as level 2 networks [2]. Chang et al. studied how to encode and compress galled trees [5]. McDiarmid et al. gave approximate formulas for the number of binary TCNs and normal networks [22]. Fuchs et al. presented generating functions for the count of labeled galled trees, normal networks and TCNs with few reticulations [8]. Cardona et al. developed an exhaustive
method for enumerating TCNs [3], which allows one to obtain the exact number of TCNs on six taxa. Additionally, the author of this paper and collaborators provided a recurrence approach for counting and enumerating galled networks on \( n \) taxa [12]. Unfortunately, no simple formulas are known for exactly counting TCNs and normal networks in terms of the number of reticulations and the number of leaves.

We make three contributions to counting TCNs and other classes of networks. First, we establish a relationship between ordered trees and binary galled trees. Using this relationship, we derive a different formula for the number of galled trees and the first formula for the number of normal galled trees in Section 3. Secondly, the concepts of tree-components and network compression were introduced by the author and his collaborators to study the tree containment problem [11, 30]. Here, we apply these concepts to study how to count TCNs. We present a simple closed formula for counting TCNs in which the child of each reticulation is a leaf. We then present a recurrence formula to count TCNs through counting and enumerating their tree-component graphs in Section 4. Additionally, by using the obtained formulas, we are able to compute the exact number of TCNs on eight taxa. Lastly, we present explicit formulas for counting phylogenetic networks with one or two reticulations in different network classes. We conclude the study by posing several research problems about counting phylogenetic networks.

2. Basic Notation.

2.1. DAGs. A directed graph consists of a finite nonempty node set \( V \) together with a specified set \( E \) of ordered pairs of nodes of \( V \). Each element of \( E \) is called an edge. A DAG is a directed graph with no loops and no directed cycles. In this study, two parallel edges with the same orientation may exist between two distinct nodes.

Let \( u \) and \( v \) be two nodes of a DAG. If \((u, v) \in E\), we say that \( u \) is a parent of \( v \) and \( v \) is a child of \( u \). The outdegree and indegree of \( u \) are defined to be the number of children and parents of \( u \), respectively. The nodes of outdegree 0 are called the leaves.

If there is a directed path from \( u \) to \( v \), \( u \) is said to be an ancestor of \( v \) and to be above \( v \); \( v \) is said to be a descendant of \( u \) and to be below \( u \). The nodes \( u \) and \( v \) are incomparable if neither is an ancestor of the other. The set of leaves below \( u \) is called the cluster of \( u \). Two trees are not identical if and only if they contain different node clusters [19].

A rooted DAG has a node of indegree 0, called the root, which is distinguished from the others. In a labeled DAG with \( n \) nodes, the integers from 1 to \( n \) are assigned to the nodes, inducing a linear ordering on the nodes. In a leaf-labeled DAG with \( k \) leaves, the integers from 1 to \( k \) are assigned to its leaves. In a ordered DAG, the children are ordered for every non-leaf node.

Two directed graphs are isomorphic if there is a one-to-one map from the node set of one graph onto that of the other which preserves the directed edges. The isomorphic map from a rooted (leaf-)labeled DAG to another preserves not only the edges but also the labeling and the root. The isomorphic map from a ordered DAG to another also preserves the ordering of the children for every node. Our object is to count non-isomorphic phylogenetic networks of different types, which are rooted DAGs used to model molecular evolution.

2.2. RPNs. A binary RPN on a finite set of taxa \( X \) is a rooted DAG with no parallel edges such that:

* the root is the unique node of indegree 0. The root is of outdegree 1.
• there are exactly \( |X| \) leaves that are labeled one-to-one with \( X \);
• non-leaf and non-root nodes are either of indegree 2 and outdegree 1, or of indegree 1 and outdegree 2; and
• each edge is directed away from the root.

For convenience, in the rest of this study, edge orientation is omitted in the graphic representation of RPNs, as illustrated in Figure 1.

The nodes of indegree 2 and outdegree 1 are called reticulations. The nodes of outdegree 2 and indegree 1 are called tree nodes. An edge \((u, v) \in E(N)\) is called a tree edge if \( v \) is either a tree node or a leaf; and the edge is called a reticulation edge if \( v \) is a reticulation. The following simple results will be used frequently. Here we omit its proof.

**Proposition 2.1.** Let \( N \) be a RPN with \( k \) reticulations on \( n \) taxa. \( N \) has \( k + n - 1 \) tree nodes and \( k + 2n - 1 \) tree edges and \( 2k \) reticulation edges.

We will adopt the following notation:

\( [n] \) the set \( \{1, 2, \cdots, n\} \), where \( n \) is a positive integer;
\( N \) a RPN on \( [n] \);
\( V(N) \) the set of nodes of a RPN \( N \);
\( R(N) \) the set of reticulations of \( N \);
\( T(N) \) the set of tree nodes of \( N \);
\( E(N) \) the set of edges of \( N \);

**2.3. Network classes.** A binary phylogenetic tree is simply a binary RPN with no reticulations.

A RPN is said to be a galled tree if every reticulation \( r \) has an ancestor \( a_r \) such that (i) \( a_r \) is a tree node, (ii) there are two edge-disjoint directed paths from \( a_r \) to \( r \) that form a cycle \( C_r \) (if edge orientation is ignored) with \( a_r \) on top and \( r \) at the bottom, and (iii) \( C_r \) and \( C_s \), as indicated in (ii), are node-disjoint for different \( r \) and \( s \). The cycles associated with reticulations under this definition will be called galls in this study. Figure 1A shows a galled tree. Note that phylogenetic trees are galled trees. Every RPN with only one reticulation is also a galled tree.

A RPN is said to be a TCN if every non-leaf node has a child that is either a tree node or a leaf (Figure 1B). Obviously, a RPN is a TCN if and only if for each non-leaf node, there is a path from it to some leaf that consists of only tree edges.

A RPN is said to be a normal network if it is a TCN and the two parents of \( r \) are incomparable for every reticulation \( r \). Figure 1C presents a normal network, whereas the TCN in Figure 1B is not a normal network.

A RPN is said to be a galled network if each reticulation \( r \) has an ancestor \( a_r \) such that Conditions (i) and (ii) under the definition of galled trees are true and (iii) all the edges of the gall \( C_r \) are tree edges except for the two edges entering \( r \). The networks in Figure 1C and D are galled networks. Clearly, gall trees are galled networks.

A RPN is said to be a reticulation-visible network if for each reticulation \( r \), there is a leaf \( \ell \) such that every path from the network root to \( \ell \) contains \( r \). Figure 1D shows a reticulation-visible network that is neither a galled network nor a TCN.

A RPN is said to be a tree-based network if it can be obtained from a phylogenetic tree by recursively inserting edges between nodes that subdivide two tree edges in the obtained network in each step.

A RPN is said to be one-component if the child of each reticulation is a leaf. The RPNs in Figure 1A, 1C and 1D are one-component networks, whereas the others are not.

Throughout this paper, we adopt the following notation:
**2.4. Tree-components and network decomposition.** Consider a RPN $N$. Let $N \in \mathcal{RN}_{n,k}$ denote the subnetwork that is obtained from $N$ by the removal of all reticulations together with the incident edges. This subnetwork is actually a forest in which each connected component consists only of tree nodes and is rooted at either the network root or the child of a reticulation. Each of these connected components is called a **tree-component** of $N$ [13, 30].

Tree-component is a useful concept for characterizing the topological structures of RPNs. A reticulation is **inner** if its two parents are in a common tree-component. It is known that each reticulation is inner for a galled network. It is also known that every tree-component of a RPN contains a leaf or the two parents of a reticulation if the network is reticulation-visible (see [30]).

It is also easy to see that a one-component network has only one non-trivial tree-component that contains all the tree nodes.

**3. Counting Galled Trees.** Let $\mathcal{GT}_{n}$ denote the set of galled trees on $[n]$ and let $\mathcal{O}_n$ be the set of rooted, ordered trees on $[n]$. In this section, we shall count (normal) galled trees in $\mathcal{GT}_n$ by establishing a many-to-many relation $m \subseteq \mathcal{O}_n \times \mathcal{GT}_n$.

Let $N \in \mathcal{GT}_{n}$. For a tree node of $N$ that is not on any gall, we let its children be $a$ and $b$. It is mapped to $m(t) = \{x, y\}$, where $x$ (resp. $y$) is a tree node with ranked children $a$ and $b$ (resp. $b$ and $a$). This is illustrated in Figure 2A.

For a tree node $g$ that is on the top of a gall $C$, we let $b$ be the unique reticulation at the bottom of $C$ and $c$ be the child of $b$. Assuming that the tree nodes are $t'_1, t'_2, \ldots, t'_p$ on one side of $C$ (clockwise from $b$ to $g$) and $t''_1, t''_2, \ldots, t''_q$ (clockwise from $g$ to $b$) on the other side, $g$ is mapped to $m(g) = \{v, w\}$, where $v$ is a tree node with ranked children $t'_1, t'_2, \ldots, t'_p, c, t''_1, t''_2, \ldots, t''_q$, whereas $w$ has the ranked children $t''_1, t''_2, \ldots, t''_q, c, t'_1, t'_2, \ldots, t'_p$, as illustrated in Figure 2B.

Using the above rules, we map a galled tree to a set of ordered trees with the same labeled leaves. Figure 2C shows how to transform a galled tree into a set of eight rooted, ordered trees with leaves labeled with integers in $[n]$. The following fact is clearly true.
Lemma 3.1. Let \( N \in \mathcal{GT}_n \) have \( r \) reticulations and \( k \) tree nodes that are not on any galls associated with reticulations. Then, replacing each of the \( r + k \) tree nodes \( t \) that are either on the top of a gall or not in any gall with either image of \( m(t) \) produces \( 2^{r+k} \) trees of \( \mathcal{O}_n \).

Conversely, we can derive a set of galled trees on \([n]\) from a rooted, ordered tree on \([n]\) by transforming a non-leaf node with \( d \) ordered children into one of \( d \) galls for \( d > 2 \), and a non-leaf node into a galled or an unordered tree node if \( d = 2 \), as shown in Figure 3. Thus, we have the following lemma.

Lemma 3.2. Let \( T \in \mathcal{O}_n \) have \( s \) nodes, each having two ordered children, and \( t \) nodes, each having \( m_1, m_2, \ldots, m_t \) ordered children, where \( m_i > 2 \) for each \( i \). \( T \) can then be transformed into \( 3^s m_1 m_2 \cdots m_t \) different galled trees on \([n]\).

Theorem 3.3. Let

\[
C = \{(k_2, k_3, \ldots, k_n) \mid n = 1 + k_2 + 2k_3 + \cdots + (n-1)k_n; k_i \geq 0, i = 2, \ldots, n\}.
\]

We then have:

\[
|\mathcal{GT}_{n,k}| = \sum_{(k_2, k_3, \ldots, k_{n-1}) \in C} \frac{(n + k_2 + \cdots + k_n - 1)!3^{k_2+k_3}4^{k_4}\cdots n^{k_n}}{k_2!k_3!\cdots k_n!(2^{k_2+k_3+\cdots+k_n})}.
\]

galled trees on \([n]\).

Proof. For any \((k_2, k_3, \ldots, k_n) \in C\), by a theorem of Erdős and Székely [6, Theorem 1], there are

\[
A = \frac{(n + k_2 + \cdots + k_n - 1)!}{k_2!(2!)^{k_2}k_3!(3!)^{k_3}\cdots k_n!(n!)^{k_n}}
\]

rooted trees on \([n]\), with \( k_i \) internal nodes having \( i \) children for \( i = 1, 2, \ldots, n \). For each internal node with \( t \) children, these children can be ordered in \( t! \) different ways. Thus, there are

\[
B = (2!)^{k_2}(3!)^{k_3}\cdots(n!)^{k_n}A = \frac{(n + k_2 + \cdots + k_n - 1)!}{k_2!k_3!\cdots k_n!}.
\]

Fig. 2. Illustration of transformation from a galled tree to a rooted, ordered tree with same labeled leaves. A and B. A mapping from a tree node to two ordered tree nodes. C. Mapping a galled tree to eight rooted, ordered tree with same labeled leaves by using the rules in A and B.
rooted, ordered trees with \( k_2 + k_3 + \cdots + k_n \) internal nodes of the prescribed degrees. By Lemma 2, we can obtain \( C = 3^{k_2}3^{k_3}4^{k_4} \cdots n^{k_n} B \) galled trees on \([n]\) from the \( B \) rooted, ordered trees.

By Lemma 1, each galled trees can be obtained from \( D = 2^{k_2+k_3+\cdots+k_n} \) rooted, ordered trees. Furthermore, we derive Eqn. (3.1) from dividing \( C \) by \( D \). □

Since each internal node with \( t \geq 3 \) ordered children can be transformed into \( t - 2 \) galls in which each side path contains at least one node, we obtain the following formula to count galled trees on \([n]\) that are also normal networks.

**Theorem 3.4.** Let

\[
C = \{(k_2, k_3, \ldots, k_n) \mid n = 1 + k_2 + 2k_3 + \cdots + (n-1)k_n; k_i \geq 0, i = 2, \cdots, n\}.
\]

There are:

\[
\sum_{(k_2, k_3, \ldots, k_{n-1}) \in C} \frac{(n + k_2 + \cdots + k_n - 1)!1^{k_2}2^{k_3} \cdots (n-2)^{k_n}}{k_2!k_3! \cdots k_n!2^{k_2+k_3+\cdots+k_n}}
\]

normal galled trees on \([n]\).

To conclude this subsection, we present a formula to count one-component galled trees, which will be used when the galled trees with 2 reticulations are counted. Recall that the child of each reticulation is a leaf for one-component galled trees.

![Fig. 3. Illustration of transformation from a rooted, ordered tree to a set of galled trees. The ordered tree has the internal nodes a and b. The node a has three children ordered from left to right. It can be mapped to one of the three possible galls (shaded in the first column). The node b has two children ordered from left to right. The node can remain as a binary node or be mapped to one of the two possible galls (shaded in the first row). Therefore, the ordered tree corresponds to nine galled trees.](image)
Proposition 3.5. Let $\mathcal{OGT}_{n,k}$ be the set of one component galled trees with $k$ reticulations on $[n]$ in which the children of the $k$ reticulation are Leaves 1, 2 and $k$, respectively, where $1 \leq k < n$ and $n \geq 3$. Then, 

\begin{align}
|\mathcal{OGT}_{n,1}| &= \frac{(2n-2)!}{2^{n-1}(n-2)!}, \\
|\mathcal{OGT}_{n,2}| &= \frac{(2n-2)!}{3 \cdot 2^{n-1}(n-3)!}, \\
|\mathcal{OGT}_{n,k}| &= \frac{1}{2^{n+k-1}} \sum_{j=0}^{k} (-1)^{k-j} \binom{k}{j} \frac{(2n+2j-2)!}{(2j)!(n+j-1)!}, 
\end{align}

where $0! = 1$.

Proof. Eqn. (3.3) and (3.4) are special cases of Eqn. (3.5) where $k$ is 1 and 2, respectively. Nevertheless, we first prove Eqn. (3.3) as a warm-up exercise.

In a RPN, two leaves are said to be in a cherry if they are adjacent to the same tree node. Consider all $\frac{(2n)!}{2^{n-1}(n-1)!}$ possible phylogenetic trees on $[n+1]$. From each of these trees such that Leaves 1 and $n+1$ are not in a cherry, we can generate a galled tree of $\mathcal{OGT}_{n,1}$ by inserting Leaf $n+1$ into the edge leading to Leaf 1 so that Leaf $n+1$ becomes a reticulation parent of Leaf 1 in the resulting galled tree (Figure 4A).

Since there are $\frac{(2n-2)!}{2^{n-1}(n-1)!}$ trees in which Leaves 1 and $n+1$ are in a cherry, there are $\frac{(2n)!}{2^{n-1}(n-1)!} - \frac{(2n-2)!}{2^{n-1}(n-1)!}$ trees in which the two leaves do not appear in a cherry.

For two trees $T_1$ and $T_2$, our galled tree generation procedure produces the same galled tree from them if $T_1$ is identical to $T_2$ after Leaf 1 and Leaf $n+1$ are interchanged. Therefore, $|\mathcal{OGT}_{n,1}| = \frac{1}{2} \left( \frac{(2n)!}{2^{n-1}!} - \frac{(2n-2)!}{2^{n-1}(n-1)!} \right) = \frac{(2n-2)!}{2^{n-1}(n-2)!}$, obtaining Eqn. (3.3).

In order to prove Eqn. (3.5), we first introduce the edge attachment operation,
illustrated in Figure 4B. Let $T$ and $T'$ be unrooted two phylogenetic trees on $L$ and $L'$, respectively, such that $L \cap L' = \emptyset$. An edge attachment operation builds a phylogenetic tree on $L \cup L'$ by adding an edge between a node that is either a node subdividing an edge of $T$ or the unique node of $T$ if $|\mathcal{V}(T)| = 1$ and another node that is either a node subdividing an edge of $T'$ or is the node of $T'$ if $|\mathcal{V}(T')| = 1$. Clearly, we can obtain a set of phylogenetic trees from a forest consisting of $k$ unrooted phylogenetic trees over distinct taxa by applying $k$ edge attachments between the original and resulting trees in such a way that each edge attachment reduces the number of the trees in the forest by 1.

We use $K(i, i')$ to denote the one-edge unrooted tree with labeled leaves $i$ and $i'$ and $P(i)$ to denote the tree with a single node labeled with $i$. Let $S(m, r)$ be the set of unrooted phylogenetic trees that are obtained by applying $m + 1$ edge attachments onto a forest $F(m, r)$ consisting of $m + 1$ unrooted trees $\{K(i, m + 1), P(0), P(j) \mid 1 \leq i \leq r, r + 1 \leq j \leq m\}$. By Theorem 2.8.3 in [23, page 39], we obtain:

$$|S(m, r)| = \frac{b(m + r + 1)}{b((m + r + 1) - (m + 1) + 2)} \prod_{i=1}^{r} |\mathcal{E}(K(i, m + i))|$$

$$= \frac{b(m + r + 1)}{b((m + r + 1) - (m + 1) + 2)}$$

$$= \frac{(2m + 2r - 2)!r!}{2^{m-1}(m + r - 1)!2r!},$$

as $|\mathcal{E}(K(i, m + i))| = 1$ and $b(k + 1) = \frac{(2k-2)!}{2^{k-1}(k-1)!}$, which is the number of unrooted phylogenetic trees on $k + 1$ taxa.

Let $k \leq n$. Let $T \in S(n, k)$ such that Leaves $i$ and $i + n$ are not in a cherry for each $i$ from 1 to $k$. We can then obtain a galled tree $G$ with $k$ reticulations on $[n]$ by rooting the tree at Leaf 0 and inserting Leaf $i + n$ into the edge leading to Leaf $i$ so that $i + m$ becomes the reticulation parent of Leaf $i$ for each $i$, as illustrated in Figure 4D.

Let $T \in S(n, k)$ in which $i$ and $i + n$ are in a cherry, where $i \leq k$. Let $c_i$ be the node that are adjacent to Leaves $i$ and $i + n$ in $T$. Removing $i$ and $i + n$ together with the edges incident to them produces a unrooted tree that can also be generated by applying edge attachment from $F' = F(n, k) + \{P(c_i)\} - \{K(i, i + n)\}$. Conversely, we can obtain a unrooted phylogenetic tree of $S(n, k)$ in which $i$ and $i + n$ form a cherry by attaching $i$ and $i + n$ below $c_i$ as its children from a unrooted tree that is built from $F'$ through edge attachment. For any subset $I$ of $[1, k]$, $S(n, k)$ contains exactly $|S(n, k - |I|)|$ unrooted phylogenetic trees in which $i$ and $i + n$ form a cherry for each $i \in I$. Since $[1, k]$ has $\binom{k}{j}$ subsets each containing $j$ integers, by Inclusion–Exclusion principle, $S(n, k)$ contains $\sum_{j=0}^{k} (-1)^{k-j} \binom{k}{j} \frac{(2n+2j-2)!j!}{2^{n+1}(n+j-1)(2j)!}$ phylogenetic trees in which no pairs of $i$ and $i + n$ form a cherry. Since each galled tree with $k$ reticulations can be generated from $2^k$ trees of $S(n, k)$, we obtain Eqn. (3.5).

**Corollary 3.6.** The number of one component galled trees with $k$ reticulations on $[n]$ is equal to $\binom{n}{k} |\mathcal{OGT}_{n,k}|$.

**Proof.** The result is derived from the fact that each subset of $k$ taxa can be the children of the $k$ reticulations in a one-component galled tree.

4. Counting TCNs. Recall that $\mathcal{T}_{n,k}$ denotes the set of TCNs with $k$ reticulations on $[n]$. We will enumerate and count the networks of $\mathcal{T}_{n,k}$ through enumerating their component graphs.
4.1. One-component TCNs. One-component TCNs can be considered as the building blocks of arbitrary TCNs, as we will see later. We use \( \mathcal{O}_{n,k} \) to denote the set of one-component TCNs with \( k \) reticulations over \([n]\). Recall that the child of each reticulation is a leaf in a network of \( \mathcal{O}_{n,k} \).

Let \( N \in \mathcal{O}_{n,k} \). For any \( r \in \mathcal{R}(N) \), \( N \ominus r \) is used to denote the network obtained from \( N \) after the removal of \( r \), its child \( c(r) \), the three edges incident to \( r \), as shown in Figure 5. In general, for any \( R = \{r_1,r_2,\cdots,r_k\} \subseteq \mathcal{R}(N) \), we define:

\[
N \ominus R = (\cdots((N \ominus r_1) \ominus r_2) \ominus \cdots) \ominus r_k).
\]

A DAG \( D \) is said to be a subdivision of a phylogenetic tree \( T \), if (i) \( \mathcal{V}(T) \subseteq \mathcal{V}(D) \), (ii) each node of \( \mathcal{V}(D) \setminus \mathcal{V}(T) \) is of indegree 1 and outdegree 1 and (iii) for each \((u,v) \in \mathcal{E}(T)\) or there is a unique path from \( u \) and \( v \) passing only nodes in \( \mathcal{V}(D) \setminus \mathcal{V}(T) \).

**Lemma 4.1.** Let \( N \in \mathcal{O}_{n,k} \) such that \( \mathcal{R}(N) = \{r_1,r_2,\cdots,r_k\} \).

(i) For any \( R \subseteq \mathcal{R}(N) \), \( N \ominus R \) is also a TCN, where there may be some degree-2 nodes.

(ii) \( N \ominus \mathcal{R}(N) \) is the subtree \( \hat{N} \) of \( N \) spanned by leaves that are the children of tree nodes.

(iii) Let \( T \) be the phylogenetic tree such that \( N \ominus \mathcal{R}(N) \) is a subdivision of \( T \). \( N \) can then be obtained from \( T \) by inserting the \( k \) reticulations of \( \mathcal{R}(N) \) together with their leaf children one by one.

**Proof.** (i) Let \( r \in \mathcal{R}(N) \). The parents of \( r \) are both tree nodes and remain in \( N \ominus r \), as we only remove \( r \) and its unique children \( c(r) \) to get \( N \ominus r \). Therefore, each tree path in \( N \) remains in \( N \ominus r \), implying that \( N \ominus r \) is a TCN. By definition, we remove reticulations of \( R \) one by one to get \( N \ominus R \) and thus \( N \ominus R \) is a TCN for any subset \( R \subseteq \mathcal{R}(N) \).

(ii) Let \( N' = N \ominus \mathcal{R}(N) \). Let \((u,v) \in \mathcal{E}(N') \). By (i), \( N' \) is a TCN and thus there is a path from \( v \) to some leaf \( \ell \) consisting of only tree edges. Since \( N' \) does not contain any reticulation, \( v \) is a tree node, \( \ell \) is not the child of any reticulation in \( N \).

![Illustration of reticulation insertions and deletions](image)

**Fig. 5. Illustration of reticulation insertions and deletions.** There are two types of insertions and deletions in tree-child networks: The added or removed reticulation straddles two tree edges (left) or is attached onto a single tree edge (right).
This implies that \((u, v)\) is an edge of the subtree spanned by the leaves that are the children of tree nodes in \(N\).

Conversely, we observe that the operation \(\ominus\) is conducted by removing only edges that are incident to the removed reticulation. Since any edge \(e\) in the subtree \(N\) is not incident to any reticulation, \(e\) is also an edge in \(N'\). Thus, \(N' = \bar{N}\).

(iii) The statement follows from the fact that the tail of any removed edge is a degree-2 node in \(N'\) that is introduced in (ii).

Conversely, we consider insertion of a reticulation with a leaf child into a one-component TCN. For such a TCN \(N\) on \(A\) such that \(A = \{a_1, a_2, \ldots, a_{n-1}\} \subset [n] \quad (m < n)\), any pair of tree edges \(\{e_1, e_2\} \subset \mathcal{E}(N)\) and \(a \not\in A\), we use \(N((e_1, e_2), \ominus, a)\) to denote the network obtained from \(N\) by inserting a reticulation \(r\), together with its child \(\text{Leaf} a\), onto \(e_1\) and \(e_2\), as shown in Figure 5 (see [31]). Here, we allow the possibility that \(e_1 = e_2\). Formally, let \(e_1 = (u_1, v_1)\) and \(e_2 = (u_2, v_2)\). In this case,

\[
\begin{align*}
\mathcal{V}(N((e_1, e_2), \ominus, a)) &= \mathcal{V}(N) \cup \{x_1, x_2, r, a\}, \\
\mathcal{E}(N((e_1, e_2), \ominus, a)) &= \mathcal{E}(N)/\{e_1, e_2\} \\
&\cup \left\{ \{x_i, (x_i, v_i), (x_i, r), (r, a) \mid i = 1, 2\}, \quad \text{if } e_1 \neq e_2, \\
&\{\{x_1, (x_1, x_2), (x_1, r), (x_2, r), (r, a)\}, \quad \text{if } e_1 = e_2, \\
\right. 
\end{align*}
\]

where \(x_1\) and \(x_2\) are the nodes that subdivide \(e_1\) and \(e_2\), respectively.

**Lemma 4.2.** Let \(N\) be a one-component TCN on \(A \subset [n]\). For any \(b \in [n]\setminus A\) and four tree edges \(e_1', e_2', e_1'', e_2''\) in \(N\), \(N((e_1', e_2'), \oplus, b) = N((e_1'', e_2''), \oplus, b)\) if and only if \(\{e_1', e_2'\} = \{e_1'', e_2''\}\).

**Proof.** Let \(p_1'\) and \(p_2'\) be the parents of the reticulation \(r'\) that are inserted into \(e_1'\) and \(e_2'\), respectively, in \(N((e_1', e_2'), \oplus, b)\). We also let \(p_1''\) and \(p_2''\) be the parents of the reticulation \(r''\) that are inserted into \(e_1''\) and \(e_2''\), respectively, in \(N((e_1'', e_2''), \oplus, b)\). Assume \(N((e_1', e_2'), \oplus, b) = N((e_1'', e_2''), \oplus, b)\). There is then an isomorphic map \(\phi\) from \(N((e_1', e_2'), \oplus, b)\) to \(N((e_1'', e_2''), \oplus, b)\) that preserves the edges and leaves. Since \(\phi\) maps \(\text{Leaf} b\) in the former to \(\text{Leaf} b\) in the latter, \(\phi(r') = r''\) and thus \(\{\phi(p_1'), \phi(p_2')\} = \{p_1'', p_2''\}\). This implies that \(e_1' = e_2'\) if and only if \(e_1'' = e_2''\).

Note that \(p_i'\) and \(p_i''\) are tree nodes and have only a parent for \(i = 1, 2\). No matter whether \(e_1'\) and \(e_2'\) are identical or not, we can further deduce that the parent and child of \(p_i'\) and \(p_i''\) are mapped to the parent and child of \(\phi(p_i')\) and \(\phi(p_i'')\). Therefore, \(\phi\) induces an auto-isomorphic map for \(N\). This proves that \(\{e_1', e_2'\} = \{e_1'', e_2''\}\). \(\square\)

For any \(B \subset [n]\setminus A\), we use \(N \oplus B\) to denote the set of all possible TCNs obtained by inserting \(k\) reticulations that each have a leaf child labeled with a unique element in \(B\). Lemma 4.2 implies that any TCN with \(k\) reticulations \(r_i\) \((1 \leq i \leq k)\) can be obtained from a phylogenetic tree spanned by the leaves that are not below any reticulations by sequentially inserting the corresponding reticulations one by one in a unique way. Moreover,

**Lemma 4.3.** Let \(T'\) and \(T''\) be two phylogenetic trees on \(A\) such that \(A \subset [n]\). For any \(B \subset [n]\setminus A\), \((T' \oplus A) \cap (T'' \oplus B) \neq \emptyset\) only if \(T' = T''\).

**Proof** Assume that \(T'\) and \(T''\) are distinct phylogenetic trees on \(A\). The fact that \(T' \neq T''\) implies that there is a node \(u\) in \(T'\) such that the cluster \(C_u\) of \(u\) is not found in \(T''\) (see [26] for example).

For any \(B\), let \(N \in T' \oplus B\). In \(N\), the hard cluster \(C_u'\) of \(u\) is equal to \(C_u \cup B'\) for some \(B' \subset B\). If \(C_u'\) appears in a TCN \(M \in T'' \oplus B\), then, \(C_u\) appears in \(M \oplus R\), where
Table 1

Counts of one-component TCNs with \( k \) reticulations on \([n]\), \(1 \leq k < n \leq 7\).

| \( k \backslash n \) | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|-----------------|---|---|---|---|---|---|---|
| 1               | 2 | 18 | 180 | 2,100 | 28,350 | 436,590 | 7,567,560 |
| 2               | 18 | 540 | 12,600 | 283,500 | 6,548,850 | 158,918,760 |
| 3               | 360 | 25,200 | 1,340,000 | 43,659,000 | 1,589,187,600 |
| 4               | 12,600 | 1,701,000 | 130,977,000 | 7,945,938,000 |
| 5               | 680,400 | 157,172,400 | 19,070,251,200 |
| 6               | 52,390,800 | 19,070,251,200 |
| 7               | 5,448,643,200 |

\( R \) is the set of reticulations whose leaf children have labels in \( B \). This implies that \( C_u \) also appears in \( T'' \), which is a contradiction. Therefore, \((T' \oplus B) \cap (T'' \oplus B) = \emptyset\) for any \( B \) if \( T' \) and \( T'' \) are distinct. \( \square \)

**Theorem 4.4.** For any \( n \) and \( k \leq n - 1 \),

\[
|O_{n,k}| = \binom{n}{k} \frac{(2n - 2)!}{2^{n-1}(n - k - 1)!}.
\]

**Proof.** Let \( N \in O_{n,k} \). \( N \ominus R(N) \) is a subdivision of one of the \( t_{n-k} = \frac{(2(n-k)-2)!}{(n-k-1)2^{n-k-1}} \) rooted phylogenetic trees, each having \( n-k \) leaves in the non-trivial tree-component of \( N \), which contains the network root. Let \( T \) be this tree. We have that \( T \) contains \( 2(n-k) - 1 \) edges (Proposition 2.1) and \( N \) is obtained from \( T \) by inserting \( k \) reticulations.

To add a reticulation \( r \) into \( T \), we either insert the two parents of \( r \) into an edge of \( T \) or insert them onto two distinct edges. Thus, a reticulation can be added into \( T \) in \( (2(n-k)-2)!/(n-k-1)2^{n-k-1} \) ways.

After the first reticulation is added, \( T \) is subdivided into a tree \( T_1 \) with \( 2(n-k) + 1 \) edges in the resulting network that has one reticulation. Therefore, the second reticulation can be added into \( T_1 \) in \( (2n-2k-1)/2 \) ways. By induction, for any \( i \), \( T \) will further be subdivided into a tree \( T_i \) with \( 2(n-k) - 1 + 2i \) edges after the first \( i \) reticulations are inserted and the \( (i+1) \)-th reticulation can be inserted in \( (2n-2k+2i-1)/(2n-2k+2i) \) ways. Therefore, according to Proposition 4.2, by inserting \( k \) reticulations one by one in \( T \), we obtain:

\[
s_{n,k} = \frac{(2(n-k)-1)(2(n-k))(2(n-k)+1)(2(n-k)+2)\ldots(2n-3)(2n-2)}{2^k}
\]

TCNs of \( O_{n,k} \). Since any \( k \) out of \( n \) leaves can be selected to be the children of the \( k \) reticulations,

\[
|O_{n,k}| = \binom{n}{k} \times t_{n-k} \times s_{n,k} = \binom{n}{k} \frac{(2n-2)!}{2^{n-1}(n - k - 1)!}
\]

The counts of \( O_{n,k} \) for \( 1 \leq k < n \leq 8 \) are given in Table 1. We remark that \( |O_{n,k}| \) increases as \( k \) increases for \( k \leq n - \sqrt{n + 1} \) and decreases as \( k \) increases for \( k > n - \sqrt{n + 1} \). This fact can be proved by considering the derivative of \( |O_{n,k}|/|O_{n,k+1}| \).
4.2. Component graph. We shall work on component graphs to count arbitrary TCNs on \([n]\). Let \(N \in \mathcal{T}_{n,k}\). Since \(N\) has \(k\) reticulations, it contains \(k + 1\) tree-components, say, \(C_0, C_1, C_2, \ldots, C_k\), where \(C_0\) is the component containing the network root and the other components are each rooted at the children of the \(k\) reticulations.

Assume that the \(k\) reticulations are \(\{r_i \mid 1 \leq i \leq k\}\) and that the child of \(r_i\) is the root of the component \(C_i\). The component graph \(G(N)\) is a direct graph that has the node set \(\{C_0, C_1, \ldots, C_k\}\) and the edge set \(\{(C_i, C_j) \mid r_j\) has a parent in \(C_i\}\). Since \(N\) is a TCN, the parents of \(r_i\) are tree nodes for each \(i\) and thus \(G(N)\) is well defined and acyclic, in which the edges are oriented away from \(C_0\). The component graph of the TCN in Figure 1b is given in Figure 6. Here, we allow double edges between a pair of components \(C_i\) and \(C_j\), which indicates that \(r_j\) is an inner reticulation and its parents are both in \(C_i\). Since \(N\) is a TCN, each component \(C_i\) contains a subset \(L_i\) of labeled leaves such that \(L_1, L_2, \ldots, L_k\) form a partition of \([n]\). Hence, the component graphs of TCNs have a one-to-one correspondence with labeled DAGs with the property that all non-root nodes are each of indegree 2, where the nodes are uniquely labeled with the nonempty parts of a partition of \([n]\).

In the rest of this subsection, we will enumerate and count the component graphs of TCNs as a class of rooted DAGs in which the nodes are uniquely labeled, all nodes except the root are of indegree 2 and two parallel edges with the same orientation between two nodes are allowed.

Let \(G\) be a rooted DAG. The height \(h(u)\) of a node \(u\) is recursively defined as:

\[
h(u) = \begin{cases} 
0 & \text{if } u \text{ is a leaf,} \\
1 + \max_{v \in \text{children}(u)} h(v) & \text{if } u \text{ is a non-leaf node.}
\end{cases}
\]

(4.2)

Since \(G\) is acyclic, the height of each node can be computed via a bottom-up approach. The level of \(G\) is defined to be one plus the height of its root, denoted by \(l(G)\). For any \(0 \leq k < l(G)\), the \(k\)-th row of \(G\) is defined to be the set of the nodes of height \(k\), denoted by \(R_k(G)\). Note that \(R_0(G)\) consists exactly of all leaves of \(G\). The properties of the node height and rows are summarized as follows (the proofs have been omitted).

**Proposition 4.5.** Let \(G\) be a rooted DAG of level 1.

(i) \(\mathcal{V}(G) = \bigcup_{0 \leq k < l} R_k(G)\) and \(R_i(G) \cap R_j(G) = \emptyset\) if \(i \neq j\).

\[\begin{array}{c}
\text{FIG. 6. The component graph of the TCN in Figure 1b. The TCN has four components (left). } C_0 \text{ consists of six internal tree nodes, Leaf 1 and Leaf 5; } C_2 \text{ contains an internal node and Leaf 3; } C_1 \text{ and } C_3 \text{ contain only a single leaf. In the component graph (right) each component is labeled by the set of leaves that appear in it.}
\end{array}\]
(ii) Let \( u \in R_h(G) \). For any edge \((u, v) \in E(G), h(v) < k \). Moreover, there is a \( v \) such that \((u, v) \in E(G) \) and \( h(v) = k - 1 \).

(iii) There is no edge between two nodes in each row.

Proposition 4.5 implies that we can construct a large rooted DAG by adding nodes row by row. For \( m \geq 1 \), we define \( D_m \) to be the set of all the rooted labeled DAGs with \( m \) nodes that may have double parallel edges and satisfy the indegree constraint:

**Indegree Constraint** Every non-root node is of indegree 2.

Clearly, \( D_1 \) contains only the graph that has an isolated node.

Let \( t, s \) and \( m \) be positive integers such that \( s < m \) and \( t < m - s \). Consider \( G \in D_{m-s} \) such that \(|R_0(G)| = t\). For convenience, we set:

\[
R_0(G) = \{u_1, u_2, \ldots, u_t\}, \quad \mathcal{V}(G)/R_0(G) = \{u_i \mid t + 1 \leq i \leq m - s\}.
\]

We can then extend \( G \) to get different rooted DAGs \( G' \) of \( D_m \) such that \(|R_0(G')| = s\) by:

- Adding \( s \) new nodes \( v_1, v_2, \ldots, v_s \), and
- Adding two directed edges \((u_a, v_i) \) and \((u_b, v_i) \) for each \( i \in [1, s] \) such that there is at least an added edge leaving \( u_i \) for each \( u_i \in R_0(G) \). Here, if \( u_a = u_b \), the two added edges become parallel edges between \( u_a \) and \( v_i \).

Figure 7 displays all eight possible extensions from a graph (blue) that consists of two parallel edges from node \( a \) to node \( b \). Furthermore, Figures S1 and S2 list all the unlabeled component graphs with at most five nodes.

**Theorem 4.6.** \( D_m \) denotes the set of labelled rooted DAGs in which the non-root nodes are of indegree 2 and double edges between two nodes are allowed. Let \( \alpha_m = |D_m| \) and \( \alpha_m(s) = |\{G \in D_m \mid |R_0(G)| = s\}| \). The counts \( \alpha_m \) and \( \alpha_m(s) \) can be computed via the following recurrence relations:

\[
\alpha_1(1) = 1, \\
\alpha_m = \sum_{1 \leq s \leq m-1} \alpha_m(s), m > 1 \tag{4.3}
\]

\[
\alpha_m(s) = \sum_{1 \leq t \leq \min(s/2, m-s-1)} \binom{m}{s} \beta(m, s, t) \alpha_{m-s}(t), \tag{4.4}
\]

where

\[
\beta(m, s, t) = \sum_{0 \leq \ell \leq t} (-1)^\ell \binom{t}{\ell} \binom{m-s-\ell+1}{2}^s \tag{4.5}
\]

**Fig. 7. Illustration of graph extension.** \( G \) (blue) consists of two parallel edges from node \( a \) to node \( b \). It can be extended into eight non-isomorphic labeled DAGs of level 3 by adding two new leaves.
and we assume \( \binom{1}{2} = 0 \).

**Proof** Both \( \alpha_1(1) = 1 \) and Eqn. (4.3) are straightforward.

To prove Eqn. (4.4), we consider how the edges are added between the new nodes \( v_i \) and the nodes in \( G \). For each new node \( v_i \), the two edges entering \( v_i \) will be added from a common node or from two different nodes \( u_j \) and \( u_k \) \((k \neq j)\). Therefore, all the \( s \) new nodes can be connected to \( G \) in \( \left( \binom{y}{2} + y \right)^s = (\frac{y + 1}{2})^s \) ways if the edges have \( y \) possible tails for \( y \leq m - s \). By the Principle of Inclusion–Exclusion, the constraint that \( u_i \) must be connected to some \( v_j \) for each \( i \leq t \) implies that the nodes in \( G \) are connected to \( u_i \)'s in \( \beta(m, s, t) \) ways.

Since each node \( u_i \) is of indegree 2, the constraint that each \( v_i \in R_0(G) \) has to be connected to at least one new node implies that \( t \leq s/2 \). Moreover, \( t \) is the number of leaves in \( G \). Since \( G \) has \( m - s \) nodes, \( t \leq m - s - 1 \), where \( m - s > 1 \). For each of the \( (\binom{n}{s}) \) labelings of the new notes \( v_i \), there are \( \alpha(m - s, t) \) DAGs to be extended. Taken together, these two facts imply the formula in Eqn. (4.5). \( \square \)

**Remarks** (a) The count \( \alpha_m \) is actually the number of all labeled rooted RPNs in which every tree node is of outdegree 2 or more and each reticulation is of exact indegree 2. These networks are called **bicombining** RPNs in literature [19, page 140].

(b) Figures S1 and S2 list actually all the unlabeled component graphs of all RPNs with zero to four reticulations. However, for a RPN that is not a tree-child network, each component of it may or may not contain any network leaves and some components may be empty if there exist adjacent reticulations. We will use this fact for counting galled networks and arbitrary RPNs with only two reticulations in Section 5.3.

### 4.3. Arbitrary TCNs.

Now we are able to enumerate all the TCNs on \([n]\) by further extending all the component graphs in \( \cup_{1 \leq m \leq n} D_m \).

An \( m \)-partition of a set is a partition of the set into exactly \( m \) non-empty parts.

Let \( 1 \leq m < n \) and \( \pi \) be a partition of \([n]\) that divides \([n]\) into \( m + 1 \) non-empty parts, say, \( \{B_i\}_{i=0}^m \). We consider all the \( \alpha_{m+1} \) graphs \( G_j \) in \( D_{m+1} \). We further assume that all the graphs in \( D_{m+1} \) have nodes labeled by integers from 0 to \( m \). We extend all \( G_j \)'s into TCNs by reversing the network compression process presented in Table 2.

**Theorem 4.7.** Let \( \gamma_n(m) \) denote the number of TCNs with \( m \) reticulations on \([n]\) and let \( \Pi_{n,m+1} \) be the set of the \((m+1)\)-partitions of \([n]\). The count \( \gamma_n(m) \) can be computed via the following formula:

\[
\gamma_n(m) = \frac{1}{2^{n+m-1}} \sum_{\{B_i\}_{i=0}^m \in \Pi_{n,m+1}} \sum_{G \in D_{m+1}} \prod_{i=0}^m \frac{2^{c_i}(2|B_i| + d_i - 2)!}{(|B_i| - 1)!},
\]

where \( d_i \) and \( c_i \) are the number of the outgoing edges and the children of each node \( v_i \) of \( G \), respectively.

**Proof.** Let \( G \in D_{m+1} \) and \( \pi = \{B_i\}_{i=0}^m \) be a \((m+1)\)-partition of \([n]\). We also let \( b_i = |B_i| \) for \( 0 \leq i \leq m \). There are \( \frac{(2b_i - 2)!}{2^{b_i}b_i!} \) phylogenetic trees \( T_i \) on \( B_i \) for expanding \( v_i \). Since each phylogenetic tree on \( B_i \) has \( 2b_i - 1 \) edges and the number of tree edges increases by 1 after an edge is inserted in the tree, the \( d_i \) edges leaving \( v_i \) can be inserted in the tree in \((2b_i - 1)(2b_i) \cdots (2b_i + d_i - 2)\) ways. However, if two edges \( e' \) and \( e'' \) leaving \( v_i \) lead to the same node, then inserting \( e' \) in an edge \( x \) and inserting \( e'' \) in another edge \( y \) produces the same tree as inserting \( e' \) in \( y \) and inserting
An algorithm for enumeration of tree-child networks.

**Table 2**

**TCN Enumeration Algorithm**

**Input:** A $(m+1)$-partition $\pi$ with partition blocks $\{B_i\}^m_0$ and $D_{m+1}$;

**Output:** All the TCNs with $m$ reticulations on $[n]$ extending from the graphs in $D_{m+1}$;

$TC(D_{m+1}, \pi) = \emptyset$; /* the set of TCNs extending from $G$s in $D_{m+1}$ using $\pi$ */

for each ordered list of $(m+1)$ phylogenetic trees $T_i$ such that $T_i$ is on $B_i$, do

for each $G \in D_{m+1}$ with $m+1$ nodes $v_i$ with label $i$ and outdegree $d_i$, do {

S0. Replace the root $v_0$ with $T_0$;

S1. If $i > 0$, change $v_i$ to a reticulation $r_i$ and attach the tree $T_i$ below $r_i$ by identifying the root of $T_i$ with the child of $r_i$;

S2. For each $i$, exhaustively select a ordered list of edges $\{e_j\}^d_i$ of $T_i$, where $e_j$'s can be identical, and do {

S2.1. Insert the tail of $j$-th edge leaving $v_i$ into the corresponding edges $e_j$, where if $e_j = e_k$, the relative positions of the tails of the two edges will be considered;

S2.2. Add the resulting TCN into $TC(D_{m+1}, \pi)$; /* multiple graphs will be added at this step */

} /* end do in S2 */

} /* end for */

} /* end for */

e'' in $x$. Let $c_i = \{x \mid x \in V(G) : (v_i, x) \in E(G)\}$. Then, there are parallel edges between $v_i$ and $d_i - c_i$ neighbors.

Since $\sum_{0 \leq i \leq m} b_i = n$ and $\sum_{0 \leq i \leq m} d_i = 2m$, we can count all the possible extensions of $G$ with $\pi$ as:

$$\gamma_n(m) = \prod_{j=0}^{m} \frac{(2b_j - 2)!}{2^{b_j - 1}(b_j - 1)!} \cdot \frac{(2b_j - 1)(2b_j) \cdots (2b_j + d_i - 2)}{2^{d_i - c_i}}$$

$$= \frac{1}{2^{n+m-1}} \prod_{j=0}^{m} \frac{2^n(2b_j + d_i - 2)!}{(b_j - 1)!},$$

implying Eqn. (4.6).\[\square\]

The number of TCNs with $k$ reticulations on $[n]$ for $1 \leq k < n$ and $3 \leq n \leq 8$ are computed and listed in Table 3.

5. Counting TCNs with Few Reticulations.

5.1. Relationships between network classes.

**Proposition 5.1.** Let $n > 2$. Then,

$$NN_{n,1} \subset TC_{n,1} = GT_{n,1} = GN_{n,1} = RV_{n,1} = RP_{n,1}.$$

**Proof.** These set proper containment and equations can be derived from the definitions of these classes.\[\square\]

**Proposition 5.2.** Let $n > 2$.

(i) $NN_{n,2} \subset TC_{n,2}$.

(ii) $GT_{n,2} \subset GN_{n,2} \subset RV_{n,2} \subset TB_{n,2}$.
Table 3
Counts of TCNs with \( k \) reticulations on \([n]\), where \( 1 \leq k < n \) and \( 3 \leq n \leq 8 \). The last row contains the total numbers of TCNs that are not phylogenetic trees, where the four counts were first obtained by Cardona et al. in [3].

| \( k/n \) | 2 | 3 | 4 | 5 | 6 | 7 | 8 |
|----------|---|---|---|---|---|---|---|
| 1        | 2 | 21| 2,805| 39,330| 623,385| 11,055,240|           |
| 2        | 42 | 1,272| 30,300| 16,418,430| 405,755,280|           |
| 3        | 2,544| 154,500| 6,494,400| 241,204,950| 8,609,378,400|           |
| 4        | 309,000| 31,534,200| 2,068,516,800| 111,376,463,200|           |
| 5        | 63,068,400| 9,737,380,800| 920,900,131,200|           |
| 6        | 19,474,761,600|           |
| 7        | 8,485,564,550,400|           |
| total    | 2 | 63 | 4,044 | 496,605 | 101,832,930 | 31,538,905,965 | 13,769,649,608,920 |

(iii) \( TC_{n,2} \cup GN_{n,2} \subset RV_{n,2} \).
(iv) \( TB_{n,2} = RPN_{n,2} \).

Proof. The relationships in (i), (ii) and (iii) are straightforward. For example, (ii) is deduced from the facts that (a) the galled network in Figure 1D is not a gall tree, (b) the network in Figure 1E is a reticulation-visible network but not a gall network and (c) the network in Figure 1F is a tree-based network but not a reticulation-visible network.

(iv) Let \( N \in RPN_{n,2} \). Since \( N \) contains only two reticulations, only one reticulation can have the other as its parent, implying that \( N \) is tree-based (see [26, Corollary 10.18, page 260] or [29, Theorem 1]).

Recall that \( 1-C \) denotes the subset of one-component networks of \( C \) for a network class \( C \).

Proposition 5.3. Let \( n \geq 3 \) and \( k \geq 1 \) such that \( n \geq k \).

(i) \( 1-NN_{n,k} \subset 1-TC_{n,k} \).
(ii) \( 1-GT_{n,k} \subset 1-TC_{n,k} \).
(iii) \( (12) \) \( 1-TC_{n,k} \subset 1-GN_{n,k} = 1-RV_{n,k} = 1-TB_{n,k} = 1-RPN_{n,k} \).

Proof. The relationships in (i), (ii) and (iii) can be derived from the definition of these classes.

5.2. Counting RPNs with one reticulation. By Proposition 5.1, the hierarchy of network classes beyond galled trees collapses from five into one. Recently, the author of this paper obtained simple formulas for the size of \( NN_{n,1} \) and the size of \( RPN_{n,1} \) (see [31]). The formula for \( RPN_{n,1} \) will be used in the next subsection. For completeness, the formula for the count of normal networks is also given below.

Proposition 5.4 ([31]). Let \( n \geq 3 \). Then,

\[
|NN_{n,1}| = \frac{(n + 2)(2n)!}{2^{n+1}n!} - 3 \cdot 2^{n-1}n!, \tag{5.1}
\]
\[
|RPN_{n,1}| = \frac{n(2n)!}{2^{n+1}n!} - 2^{n-1}n!. \tag{5.2}
\]

We remark that explicit formulas for the number of unrooted and rooted networks with one reticulation were presented in [2]. But the formulas for \(|RPN_{n,1}|\) presented here is much simpler than theirs [2, Theorem 6].
5.3. Counting RPNs with two reticulations. Proposition 5.2 implies that all the six network classes defined in Section 2.3 are distinct. This raises different counting problems for RPNs with two reticulations. In the rest of this section, we will answer three of them.

Lemma 5.5. For \( n \geq 2 \),

\[
\sum_{k=1}^{n} \binom{2k}{k} \frac{k}{2^{2k}} = \frac{(2n+1)!}{3 \cdot 2^{2n}(n-1)!},
\]

(5.3)

\[
\sum_{k=1}^{n-1} \binom{2k}{k} \binom{2n-2k}{n-k} k(n-k) = n(n-1)2^{2n-3},
\]

(5.4)

Proof. Eqn. (5.3) is trivial for \( n = 1 \). Assuming that it is true for \( n - 1 \), we then have:

\[
\sum_{k=1}^{n} \binom{2k}{k} \frac{k}{2^{2k}} = \frac{(2(n-1) + 1)!}{3 \cdot 2^{2(n-1)}(n-1)!} + \binom{2n}{n} \frac{n}{2^{2n}} = \frac{(2n+1)!}{3 \cdot 2^{2n}(n-1)!}.
\]

This proves Eqn. (5.3).

Let \( f(x) = \sum_{n \geq 0} \binom{2n}{n} x^n \). Then, \( f(x) = (1 - 4x)^{-1/2} \) (see [25, page 52]). Multiplying \( f'(x) \) by \( x \), we have:

\[
x f'(x) = \sum_{n \geq 0} \binom{2n}{n} n x^n,
\]

and

\[(x f'(x))^2 = \sum_{n \geq 0} \left( \sum_{k=0}^{n} \binom{2k}{k} \binom{2n-2k}{n-k} k(n-k) \right) x^n.
\]

On the other hand,

\[(x f'(x))^2 = x^2 \left( 2(1 - 4x)^{-3/2} \right)^2 = \sum_{n \geq 0} \binom{2+n}{2} 4^{n+1} x^{n+2}.
\]

Identifying the coefficient of \( x^n \) in these two forms, we obtain Eqn. (5.4).

Theorem 5.6. Let \( n > 2 \). The number of tree-child networks with two reticulations on \( n \) taxa is

\[
|TC_{n,2}| = \frac{n!}{2^n} \sum_{j=1}^{n-2} \binom{2j}{j} \binom{2n-2j}{n-j} \frac{j(2j+1)(2n-2j-1)}{2n-2j-1} + n(n-1)n!2^{n-3} - \frac{(2n-1)!n}{3 \cdot 2^{n-1}(n-2)!}.
\]

Proof. There are three different component graphs (the third to fifth graphs in Figure S1) for TCNs with two reticulations, called \( G_3, G_4 \) and \( G_5 \). Let \( A_i \) be the number of TCNs having \( G_i \) as their component graph for \( i = 3, 4, 5 \).

Consider a TCN such that its component graph is \( G_3 \). The structure of \( G_3 \) suggests that the top tree-component of the TCN is a one-component TCN with a fixed reticulation, whereas the bottom two tree-components are both a phylogenetic tree with the leaves contained in these components. If the top tree-component contains \( j \)
network leaves, the bottom tree-components correspond to a forest of two phylogenetic
trees on \( n - j \) taxa and hence there are \( t_{n-j} \) possibilities in total, where \( t_{n-j} \) is the
number of all the phylogenetic trees with \( n - j \) taxa. Applying the same argument as
in the proof of Theorem 4.7 to the top tree-component, we obtain:

\[
A_3 = \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j)!}{2^{j+1}(j-1)!} \cdot \frac{(2n-2j-2)!}{2^{n-j-1}(n-j-1)!} \cdot \frac{(2n-2j)!}{(n-j-1)!(n-j)!}
\]

Consider a TCN such that \( G_4 \) is its component graph. We have then that the
bottom tree-component of the TCN is a phylogenetic tree with at least one leaf, the
middle tree-component is a phylogenetic tree with \( k + 1 \) leave if it contains \( k \) leaves.
Thus, if the top tree-component contains \( j \) leaves, the bottom two tree-components
is essentially a phylogenetic tree with \( n - j \) leaves with an edge from the top tree-
component being inserted into one of \( 2n - 2j - 2 \) tree edges that are not adjacent to
the top reticulation node. Thus,

\[
A_4 = \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j+1)!}{2^{j+1}(j-1)!} \cdot \frac{(2n-2j-2)!}{2^{n-j-1}(n-j-1)!} \cdot \frac{(2n-2j)!}{(n-j-1)!(n-j)!}
\]

where in the first row, the first term is the number of possibilities for the top tree-
component, the second term is the number of possibilities for the tree structure
contained in the bottom two tree-components and the third term is the number of
possibilities of forming the lower reticulation by inserting the fixed leave of the top
tree-component into an edge in the bottom two tree-components.

Consider a TCN such that its component graph is \( G_5 \). The bottom two tree
components form a TCN with one reticulation, whereas the top component is a one-
component TCN with a fixed reticulation. Thus, by Proposition 5.4 and Lemma 5.5,
we have:

\[
A_5 = \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j)!}{2^{j+1}(j-1)!} \cdot \frac{(n-j)(2n-2j)!}{2^{n-j}(n-j)!} - 2^{n-j-1}(n-j)!
\]

\[
= \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j)!}{2^{j+1}(j-1)!} \cdot \frac{(2n-2j)!}{2^{n-j}(n-j)!} - \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j)!}{2^{j+1}(j-1)!} \cdot 2^{n-j-1}(n-j)!
\]

\[
= \frac{n!}{2^n} \sum_{j=1}^{n-2} \binom{j}{j} \frac{(2n-2j)!}{n-j} \cdot j(n-j) - n!2^{n-1} \sum_{j=1}^{n-2} \binom{j}{j} \cdot \frac{(2j)!}{4j!(j-1)!}
\]

\[
= \frac{n!}{2^n} \sum_{j=1}^{n-2} \binom{j}{j} \frac{(2n-2j)!}{n-j} \cdot j(n-j) - n!2^{n-1} \sum_{j=1}^{n-2} \binom{j}{j} \cdot \frac{(2j)!}{4j!(j-1)!}
\]

\[
= \frac{n(n-1)n!2^{n-3} - (2n-1)n}{3 \cdot 2^{n-1}(n-2)!}
\]

Summing the above three equations, we obtain the total number of TCNs with
two reticulations on $n$ taxa:

$$\left| T_{n, 2} \right| = A_3 + A_4 + A_5$$

$$\begin{align*}
&= \frac{n!}{2^n} \sum_{j=1}^{n-2} \frac{(2j + 2)!}{j!(j - 1)!} \frac{(2n - 2j - 2)!}{(n - j)!(n - j)!} \\
&\quad + \frac{n!}{2^{n-1}} \sum_{j=1}^{n-2} \frac{(2j + 1)!}{j!(j - 1)!} \frac{2(n - j - 1)(n - j)(2n - 2j - 2)!}{(n - j)!(n - j)!} + A_5 \\
&= \frac{n!}{2^n} \sum_{j=1}^{n-2} \binom{2j}{j} \binom{2n - 2j}{n - j} \frac{(2j + 1)(2n - j - 1)}{2n - 2j - 1} + A_5.
\end{align*}$$

The proposition is proved. 

Proposition 5.7. Let $n \geq 2$ and let $G_{n, 2}$ be the set of galled networks with two reticulations on $n$ taxa. Then,

$$\left| G_{n, 2} \right| = \frac{n!}{2^n} \sum_{j=0}^{n-2} \binom{2j}{j} \binom{2n - 2j}{n - j} \frac{(j + 1)^2(2j + 3)}{(n - j)(2n - 2j - 1)}$$

$$\quad + n(n - 1)n!2^{n-3} - \frac{(2n - 1)!n}{3 \cdot 2^{n-1}(n - 2)!}$$

Proof. Since the component graph of a galled network with two reticulations is a tree with three nodes. Thus, the component tree is either $G_3$ or $G_5$ in Figure S1. However, as we remarks in Section 4.2, unlike a TCN, a component may not contain any leaf if it is not at the bottom for a galled network.

For a galled network with $G_3$ as its component graph, the top tree-component is a 1-component network with two distinguished reticulations that may or may not contain network leaves. Additionally, each of its two bottom components is a phylogenetic tree with at least one leaf, equivalent to a phylogenetic tree with them as left and right subtrees. Therefore, by Eqn (5.5), the number of galled networks for this
Consider a galled network. If its component graph is \( G \), the top tree-component is then a 1-galled network with a distinguished reticulation that contains at least one network leaf; and the bottom tree-components form a galled network with one reticulation. Since every TCN is also a galled tree if it contains only one reticulation, the number of galled networks with two reticulations that have \( G \) as the component graph is equal to \( A_3 \), that was calculated in the proof of Theorem 5.6.

Summing \( B_3 \) and \( A_3 \), we obtain the formula.

**Proposition 5.8.** Let \( n \geq 3 \) and \( \mathcal{GT}_{n,2} \) be the set of galled trees with two reticulations on \( n \) taxa. Then,

\[
|\mathcal{GT}_{n,2}| = \frac{n!}{3 \cdot 2^n} \sum_{j=1}^{n-2} \binom{2j}{j} \binom{2n-2j}{n-j} \frac{j(j+1)(2j+1)}{(2n-2j-1)} + n(n-1)n2^{n-3} - \frac{(2n-1)n}{3 \cdot 2^{n-1}(n-2)!}.
\]

**Proof.** Since galled trees are galled networks, the component graph of each galled tree with two reticulations are either \( G_3 \) and \( G_5 \) in Figure S1. Since a galled tree is also a TCN, a component of a galled tree contains at least one network leaf.

For a galled tree with \( G_3 \) as its component graph, its top tree-component is a 1-galled tree with two distinguished reticulations; and each of its bottom two tree-components is a phylogenetic tree with at least one leaf. Therefore, by Eqn. (3.4), the number of galled trees for this case is:

\[
C_3 = \sum_{j=1}^{n-2} \binom{n}{j} \frac{(2j+2-2)!}{3 \cdot 2^j + 2 - 1} \frac{(2n-2j)!}{(j+2-3)!} \frac{2n-j-2)!}{2^{n-j-1}(n-j-1)!}.
\]

Consider a galled tree. If its component graph is \( G_5 \), the top tree-component is then a 1-galled tree with a distinguished reticulation that contains at least one network leaf; and the bottom two tree-components form a galled tree with one reticulation. Since every TCN is also a galled tree if it contains only one reticulation, the number of galled trees for this case is equal to \( A_5 \), that was calculated in the proof of Theorem 5.6.

Summing \( C_3 \) and \( A_5 \), we obtain the count result.

Lastly, we point out that the following counting problems are open:

- How to count normal networks with two reticulations on \( n \) taxa?
- How to count arbitrary RPNs with two reticulations on \( n \) taxa?
5.4. Counting one-component RPNs. Corollary 3.6 presents a formula for the count of one-component galled trees, while Theorem 4.4 presents a formula for the count of one-component TCNs.

Additionally, by Proposition 5.3, the hierarchy of network classes beyond one-component galled networks collapses from four into one. Since $1-RPN_{n,k} = 1-GN_{n,k}$, the count $a_{n,k}$ of arbitrary one-component RPNs can be calculated via the following recurrence formula [12, Theorem 3]:

$$a_{n,k+1} = \frac{(n-k)}{(k+1)}(n+1-k)(a_{n,k} + a_{n,k-1}) + \frac{n!(n-k)}{2(k+1)} \sum_{j=1}^{k} \binom{2j}{j} \frac{(n+1-j)a_{n-j,k-j} - (n+1-k)a_{n+1-j,k-j}}{2^{j}(n+1-j)!}. $$

Lastly, the following problem is open:

- How to count one-component normal networks with $k$ reticulations on $n$ taxa?

6. Conclusion. We have presented new approaches and formulas for counting TCNs, galled trees and galled networks. In addition to the problems posed in Section 5, the following questions are also open for future study:

- Is there a simple closed or recurrence formula for the number of TCNs?
- Is there a simple closed or recurrence formula for the number of galled trees?
- Is there a simple closed or recurrence formula for the number of normal networks?

New approaches seem to be needed to answer these open problems.

In Section 4, we have shown that a TCN is an expansion of a rooted, labeled DAG through the replacement of nodes with TCNs in which the child of each reticulation is a leaf. We also prove that the replacement can be obtained from phylogenetic trees by insertion of reticulations with a leaf child. Are these structural characterizations of TCNs useful in the study of other aspects of TCNs?

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Fig. S1. **List of 18 unlabeled component graphs with 1 to 4 nodes.** The graphs are listed in increasing order according to level, in which the nodes are arranged row by row. The number below each structure is the number of corresponding labeled component graphs.
Fig. S2. List of 82 unlabeled component graphs with 5 nodes. The graphs are listed in increasing order according to level. The nodes of each graph are arranged row by row. The number below each structure is the number of corresponding labeled component graphs. There are 10 structures in all but the last row.