All that Glisters is not Galled

Francesc Rosselló

and Gabriel Valiente

1 Department of Mathematics and Computer Science, University of the Balearic Islands, E-07122 Palma de Mallorca, cesc.rossello@uib.es

2 Algorithms, Bioinformatics, Complexity and Formal Methods Research Group, Technical University of Catalonia, E-08034 Barcelona, valiente@lsi.upc.edu

Abstract. Galled trees, evolutionary networks with isolated reticulation cycles, have appeared under several slightly different definitions in the literature. In this paper we establish the actual relationships between the main four such alternative definitions: namely, the original galled trees, level-1 networks, nested networks with nesting depth 1, and evolutionary networks with arc-disjoint reticulation cycles.

1 Introduction

The extension of traditional phylogenetic methods and tools to deal with reticulate evolution is hindered by the computational complexity of phylogenetic reconstruction. Several techniques such as parsimony and likelihood have been carried over from phylogenetic trees to networks [10, 16, 17, 23], but when it comes to exact methods for phylogenetic reconstruction, the hardness of reconstructing an evolutionary network with as few reticulations as possible for a given set of sequences was soon established [6, 26, 27].

Under suitable constraints on reticulation cycles, however, the latter problem can be solved in polynomial time. For instance, the so-called galled trees [6], evolutionary networks with disjoint reticulation cycles, can be reconstructed in time polynomial in the size of the sequences and, when they exist, contain the smallest possible number of reticulations that explain the evolutionary history of the given set of sequences under mutation and recombination, with the assumption of no back or recurrent mutations [4, 6–8].

The “disjoint reticulation cycles” condition for galled trees has appeared several times and in different guises in the literature. Up to our knowledge, it was first introduced as the condition C2 in [18, 26] and in the definition of perfect phylogenetic networks with recombination in [27]. Gusfield et al’s original definition of (the topology of a) galled tree is as a rooted DAG with all hybrid nodes of in-degree 2 and without nodes belonging to two reticulation cycles. In this definition, the restriction to hybrid nodes of in-degree 2 is imposed by their semantics: they represent very specific recombination operations of pairs of sequences. Although the original definition of galled tree imposes that reticulation cycles are disjoint at the level of nodes, it has been realized that their combinatorial analysis also works if they are only required to be disjoint at the level of edges [11]: lacking of a
specific term for the resulting networks, we shall call them here *weakly galled trees*,
to distinguish them from the original galled trees.

Soon later, Jansson and Sung [13, 15] introduced the *nested networks* and they claimed
that the nested networks of *nesting depth 1* (which, for simplicity, we shall abbreviate henceforth as *1-nested networks*) were the same as the galled trees in the sense of [6]. 1-nested networks, as defined in *loc. cit.*, are bijectively leaf-labelled rooted DAGs with some restrictions on the degrees of the nodes (namely, the tree nodes, including the root, have out-degree 0 or 2, and the hybrid nodes have in-degree 2 and out-degree 0, 1 or 2) and where no node is intermediate in reticulation cycles for different hybrid nodes. Also, Jansson, Sung and collaborators defined *level-k networks* [3, 14] as bijectively leaf-labelled rooted DAGs, with the same restrictions on the degrees of the nodes as in 1-nested networks, and where each biconnected subgraph contains at most \( k \) hybrid nodes, and they also claimed that level-1 networks were the galled trees. The restrictions on the degrees of the nodes in 1-nested or level-1 networks have no semantical meaning, being necessary only to guarantee that the reconstruction algorithms proposed in those papers run in polynomial-time. Thus, it is plausible that, in the future, these restrictions can be relaxed, if new algorithms using other kinds of data produce in polynomial time networks satisfying the defining conditions of 1-nested or level-1 networks, but with tree nodes of out-degree greater than 2 or hybrid nodes of in-degree greater than 2 (corresponding to combinations of mutations or recombinations, respectively, where the order of the events cannot be ascertained exactly [20]). For this reason, we do not include in our definitions of 1-nested and level-1 networks any restriction on the nodes’ degrees.

Under one name or the other, galled trees have fostered much research on phylogenetic network structure [5, 21], tight bounds on the number of reticulations [9, 22], and reconstruction algorithms [2, 6, 12, 14, 19, 24]. The goal of this paper is to study the actual relationship among galled trees, weakly galled trees, 1-nested networks, and level-1 networks, establishing in particular to which extent 1-nested networks and level-1 networks are actually galled trees. Among other things, we prove that, under the “hybrid nodes of in-degree 2” restriction, the 1-nested networks are exactly the weakly galled trees, and that the class they define strictly contains the level-1 networks, which, on their turn, are strictly more general than the galled trees. However, in the fully resolved case, all four definitions describe exactly the same networks.

2 Preliminaries

By an *evolutionary network* on a set \( S \) of *taxa* we simply mean a rooted DAG with its leaves bijectively labeled in \( S \).

A *tree node* of an evolutionary network \( N = (V, E) \) is a node of in-degree at most 1, and a *hybrid node* is a node of in-degree at least 2. A *tree arc* (respectively, a *hybridization arc*) is an arc with head a tree node (respectively, a hybrid node).
A node $v \in V$ is a child of $u \in V$ if $(u, v) \in E$; we also say in this case that $u$ is a parent of $v$. We denote by $u \rightarrow v$ any path in $N$ with origin $u$ and end $v$. Whenever there exists a path $u \rightarrow v$, we shall say that $v$ is a descendant of $u$ and also that $u$ is an ancestor of $v$. A path $u \rightarrow v$ is non-trivial when $u \neq v$: in this case, we say that $v$ is a proper descendant of $u$ and that $u$ is a proper ancestor of $v$. A minimal common ancestor (mca, for short) of a pair of nodes $u, v$ is a common ancestor of $u$ and $v$ that is not a proper ancestor of any other common ancestor of them.

We shall say that an evolutionary network is 2-hybrid when its hybrid nodes have in-degree 2, hybrid-1 when its hybrid nodes have out-degree 1, semibinary when its hybrid nodes have in-degree 2 and out-degree 1, and binary, or fully resolved, when it is semibinary and its internal tree nodes have out-degree 2.

Two paths in an evolutionary network are said to be internally disjoint when they have disjoint sets of intermediate nodes. A reticulation cycle for a hybrid node $h$ is a pair of internally disjoint paths ending in $h$ and with the same origin. Each one of the paths forming a reticulation cycle for a node $h$ is called generically a merge path for $h$, their common origin is called the split node of the reticulation cycle, and the hybrid node $h$, the end of the reticulation cycle. The intermediate nodes of a reticulation cycle are the intermediate nodes of the merge paths forming it.

**Remark 1.** Let $h$ be a hybrid node and let $u$ and $v$ be two different proper ancestors of it such that the paths $u \rightarrow h$ and $v \rightarrow h$ have only their end $h$ in common. Let $w$ be a mca of $u$ and $v$. If $w \neq u, v$ (which in particular implies that $u$ and $v$ are not connected by a path), then the paths $w \rightarrow u$ and $w \rightarrow v$ have only their origin in common, and then the concatenations $w \rightarrow u \rightarrow h$ and $w \rightarrow v \rightarrow h$ define a reticulation cycle. If, on the contrary, $w$ is one of the nodes $u, v$, say $w = u$, then $u$ is an ancestor of $v$ and the only mca of $u$ and $v$. In this case there are two possibilities. If there exists some path $u \rightarrow v$ internally disjoint from $u \rightarrow h$, then the paths $u \rightarrow h$ and $u \rightarrow v \rightarrow h$ define a reticulation cycle for $h$, with split node $u$. But if there does not exist any path $u \rightarrow v$ internally disjoint from $u \rightarrow h$, and if $w$ is the last node in the path $u \rightarrow h$ that is an ancestor of $v$, then the subpath $w \rightarrow h$ of $u \rightarrow h$ and the path $w \rightarrow v \rightarrow h$ form a reticulation cycle, with split node $w$.

A straightforward consequence of this observation is the following lemma, which will be used several times in the next sections.

**Lemma 1.** If an evolutionary network contains a hybrid node $h$ and two non-trivial paths $v_1 \rightarrow h$ and $v_2 \rightarrow h$ with only their end $h$ in common, then either $v_1$ and $v_2$ are intermediate nodes in a reticulation cycle for $h$, or one of the nodes $v_1, v_2$ is intermediate in a reticulation cycle for $h$ whose split node is a descendant of the other node. \qed

By restricting the possible type of intersections between reticulation cycles, we obtain different types of evolutionary networks:
An evolutionary network is a *galled tree* [6] when every pair of reticulation cycles have disjoint sets of nodes.

An evolutionary network is a *weakly galled tree* when every pair of reticulation cycles have disjoint sets of arcs.

An evolutionary network is *1-nested* when every pair of reticulation cycles with different ends have disjoint sets of intermediate nodes.

The last definition deserves some context. Jansson and Sung [13, 15] define an evolutionary network to be *nested* when, for every pair of hybrid nodes \( h_1, h_2 \), one of the following three conditions holds:

- Every merge path for \( h_1 \) and every merge path for \( h_2 \) are internally disjoint.
- Every merge path for \( h_1 \) is a subpath of some merge path for \( h_2 \).
- Every merge path for \( h_2 \) is a subpath of some merge path for \( h_1 \).

Then, they define a nested evolutionary network to have *nesting depth* \( k \) when every node is an intermediate node of reticulation cycles for at most \( k \) hybrid nodes. Now, notice that the nesting depth 1 condition implies the nested condition (because every pair of merge paths for different hybrid nodes will be internally disjoint), and therefore the nested networks with nesting depth 1 are exactly the evolutionary networks where no node is intermediate in reticulation cycles for more than one hybrid node, which are the networks we have dubbed 1-nested.

A subgraph of an undirected graph is *biconnected* when it is connected and it remains connected if we remove any node and all edges incident to it. A subgraph of an evolutionary network \( N \) is said to be *biconnected* when it is so in the undirected graph associated to \( N \). Every arc in an evolutionary network is a biconnected subgraph. Every reticulation cycle also induces a biconnected subgraph.

**Remark 2.** If a pair of nodes in an evolutionary network \( N \) belong to a biconnected subgraph with more than 2 nodes, then they must belong to some minimal\(^4\) cycle contained in the corresponding biconnected subgraph of the undirected graph associated to \( N \). This minimal cycle will correspond in \( N \) to a sequence of \( 2k \) (directed) different paths

\[
v_1 \rightsquigarrow h_1, v_1 \rightsquigarrow h_2, v_2 \rightsquigarrow h_2, v_2 \rightsquigarrow h_3, \ldots, v_k \rightsquigarrow h_k, v_k \rightsquigarrow h_1
\]

where \( h_1, \ldots, h_k \) are pairwise different hybrid nodes, \( v_1, \ldots, v_k \) are pairwise different nodes, and the only possible intersection between a pair of such paths is to share the origin or the end in the way indicated by the notations. To simplify the language, we shall call such a sequence of paths in \( N \) a *minimal undirected cycle*.

An evolutionary network is *level-\( k \) \([3, 14, 25]\)* when no biconnected subgraph of it contains more than \( k \) hybrid nodes. Thus, a *level-1 network* is an evolutionary network

\(^4\)By a *minimal cycle* \( (v_0, v_1, \ldots, v_k, v_0) \) we mean a cycle such that the nodes \( v_0, v_1, \ldots, v_k \) are pairwise different.
network where no biconnected subgraph contains more than 1 hybrid node. In particular, the minimal undirected cycles in a level-1 network are reticulation cycles with split node of tree type and no hybrid intermediate node.

**Remark 3.** It is clear from the definitions that every galled tree is a weakly galled tree. The converse implication is false: see Fig. 1.

![Fig. 1. Two weakly galled trees that are not galled trees.](image)

Galled trees were originally defined as being 2-hybrid, because their hybrid nodes represented recombinations of pairs of sequences [6]. Nevertheless, it turns out that the condition of having arc-disjoint reticulation cycles implies that all hybrid nodes must have in-degree 2.

**Lemma 2.** *Every weakly galled tree (and hence every galled tree) is 2-hybrid.*

**Proof.** If an evolutionary network $N$ contains some hybrid node $h$ with three different parents $a, b, c$, then it contains some reticulation cycle for $h$ with merge paths ending in $(a, h)$ and $(b, h)$, and some other reticulation cycle with merge paths ending in $(b, h)$ and $(c, h)$. These reticulation cycles share the arc $(b, h)$, which shows that $N$ is not a weakly galled tree.

Unlike galled trees, 1-nested and level-1 evolutionary networks need not be 2-hybrid: see Fig. 2.

### 3 Results for Arbitrary Networks

In this section we investigate the relationship between level-1 networks and 1-nested networks when no restriction on the in-degrees of hybrid nodes in the networks is imposed.
Fig. 2. An evolutionary network that is 1-nested and level-1 but not 2-hybrid.

**Proposition 1.** Every level-1 network is 1-nested.

**Proof.** Let $N$ be a level-1 evolutionary network and assume that it contains some node $v$ that is intermediate in reticulation cycles for two different hybrid nodes $h_1$ and $h_2$. The node $v$ must be of tree type, because otherwise the reticulation cycles of $h_1$ and $h_2$ would be biconnected subgraphs of $N$ with more than one hybrid node, which is forbidden in level-1 networks. Let $w$ be the only parent of $v$ ($v$ cannot be the root, because it is intermediate in reticulation cycles). Then the arc $(w, v)$ must belong to the reticulation cycles for $h_1$ and $h_2$ that contain $v$. This implies that the union of these two reticulation cycles is a biconnected subgraph of $N$, against the assumption that $N$ is level-1.

The converse implication is in general false: network (b) in Fig. 1 is 1-nested, but not level-1. Actually, that counterexample captures the only pathology that can prevent a 1-nested network from being level-1, as Theorem 1 below shows. To prove it, we shall use the following lemma.

**Lemma 3.** In a 1-nested network, no reticulation cycle contains an intermediate hybrid node.

**Proof.** Let $N$ be a 1-nested network, and assume that a hybrid node $h_1$ is intermediate in a reticulation cycle $C$ for a hybrid node $h_2$, and let $P : h_1 \rightsquigarrow h_2$ be the subpath of the corresponding merge path. Let $u$ be the split node of the reticulation cycle $C$, and let $P_1 : u \rightsquigarrow h_1 \rightsquigarrow h_2$ and $P_2 : u \rightsquigarrow h_2$ be the merge paths of this reticulation cycle. Let now $v_2$ be a parent of $h_1$ that is not the node preceding $h_1$ in the path $P_1$.

Assume that $v_2$ belongs to the path $P_1$. In this case the subpath $v_2 \rightsquigarrow h_1$ of $P_1$ and the arc $(v_2, h_1)$ form a reticulation cycle $C'$ for $h_1$, and the node preceding $h_1$ in the path $P_1$ will be intermediate in the reticulation cycles $C'$ for $h_1$ and $C$ for $h_2$, against the assumption that $N$ is 1-nested.

Assume now that $v_2$ belongs to the path $P_2$: since we have already discarded the possibility that $u = v_2$, and $h_2 \neq v_2$ because $N$ is acyclic, it will be intermediate in $P_2$. In this case, the subpath $u \rightsquigarrow h_1$ of $P_1$ and the concatenation $u \rightsquigarrow v_2 \rightarrow h_1$...
of the subpath \( u \sim v_2 \) of \( P_2 \) and the arc \((v_2, h_1)\) form a reticulation cycle \( C' \) for \( h_1 \), and \( v_2 \) is intermediate in the reticulation cycles \( C' \) for \( h_1 \) and \( C \) for \( h_2 \), which again contradicts the assumption that \( N \) is 1-nested.

So, \( v_2 \) does not belong to the paths \( P_1 \) or \( P_2 \). Let \( v \) be a mca of \( v_2 \) and \( u \). We must distinguish now several cases, in all of which we obtain a node that is intermediate in reticulation cycles for \( h_1 \) and \( h_2 \), contradicting the assumption that \( N \) is 1-nested:

- If \( v \neq v_2, u \), then it defines a reticulation cycle \( C' \) for \( h_1 \) with split node \( v \) and merge paths \( P_3 : v \sim v_2 \rightarrow h_1 \) and \( P_4 : v \sim u \sim h_1 \). In this case, since \( v_2 \) is neither an ancestor nor a descendant of \( u \) (because \( v \neq v_2, u \)), the paths obtained by concatenating, on the one hand, the paths \( P_3 \) and \( P \) and, on the other hand, the subpath \( v \sim u \) of \( P_4 \) and the path \( P_2 \), form a new reticulation cycle \( C'' \) for \( h_2 \), with split node \( v \). Therefore, \( v_2 \) is an intermediate node in the reticulation cycles \( C' \) for \( h_1 \) and \( C'' \) for \( h_2 \).

- If \( v = v_2 \), then the arc \((v_2, h_1)\) and the path \( P_3 : v \sim v_2 \sim h_1 \) form a reticulation cycle \( C' \) for \( h_1 \). In this case, \( v_2 \) is also the split node of a reticulation cycle \( C'' \) for \( h_2 \), with merge paths on the one hand the concatenation of the arc \((v_2, h_1)\) and the path \( P \) and, on the other hand, the concatenation of the subpath \( v_2 \sim u \) of \( P_3 \) and the path \( P_2 \). In this way, \( u \) turns out to be intermediate in the reticulation cycles \( C' \) for \( h_1 \) and \( C'' \) for \( h_2 \).

- If \( v = u \) and \( v_2 \) is not a descendant of any intermediate node in the subpath \( u \sim h_1 \) of \( P_1 \), then \( u \) is the split node of a reticulation cycle \( C' \) for \( h_1 \), with merge paths \( P_3 : u \sim h_1 \) (the corresponding subpath of \( P_1 \)) and \( P_4 : u \sim v_2 \rightarrow h_1 \). Now, the subpath \( u \sim v_2 \) of \( P_4 \) may have more nodes in common with \( P_2 : u \sim h_2 \) than the origin. Let \( w \) be the last node in \( P_2 \) that appears in the path \( u \sim v_2 \). Since \( w \neq v_2 \) (because we already know that \( v_2 \) does not belong to \( P_2 \)), the subpath \( w \sim h_2 \) of \( P_2 \) and the concatenation of the subpath \( w \sim v_2 \sim h_1 \) of \( P_4 \) with \( P \) define a reticulation cycle \( C'' \) for \( h_2 \), and \( v_2 \) is intermediate in this reticulation cycle for \( h_2 \) as well as in the reticulation cycle \( C' \) for \( h_1 \).

- If \( v = u \) but \( v_2 \) is a proper descendant of some intermediate node in the subpath \( u \sim h_1 \) of \( P_1 \), then \( w \) be the last intermediate node in \( u \sim h_1 \) with this property: in this case, \( w \) is the split node of a reticulation cycle \( C' \) consisting of the merge paths \( w \sim v_2 \rightarrow h_1 \) and the subpath \( w \sim h_1 \) of \( P_1 \). Now, the paths \( w \sim v_2 \) and \( P_2 \) may have some node in common, which leads to two possibilities:
  - If the paths \( w \sim v_2 \) and \( P_2 \) are disjoint, then the path \( P_2 \) and the concatenation of the subpath \( u \sim w \) of \( P_1 \) with the path \( w \sim v_2 \rightarrow h_1 \) followed by \( P \) form a reticulation cycle \( C'' \) for \( h_2 \) that has \( v_2 \) as an intermediate node, and \( v_2 \) was already an intermediate node of the reticulation cycle \( C' \) for \( h_1 \).
  - If the paths \( w \sim v_2 \) and \( P_2 \) are not disjoint, let \( u' \) be the last node in \( P_2 \) that also belongs to \( w \sim v_2 \). Since \( u' \neq v_2 \), because \( v_2 \) does not belong to \( P_2 \), the subpath \( w' \sim h_2 \) of \( P_2 \) and the concatenation of the subpath \( w' \sim v_2 \)
of $w \leadsto v_2$ with the arc $(v_2, h_1)$ and the path $P$ yields a reticulation cycle $C''$ for $h_2$ with split node $w'$. Then, $v_2$ is intermediate in this reticulation cycle for $h_2$ as well as in the reticulation cycle $C'$ for $h_1$.

Thus, all possible situations arising when a hybrid node is intermediate in a reticulation cycle lead to a contradiction in 1-nested networks.

**Theorem 1.** The level-1 networks are exactly the 1-nested networks without hybrid split nodes.

**Proof.** Every level-1 network is 1-nested by Proposition 1, and it has no hybrid split node, because a reticulation cycle with hybrid split node induces a biconnected subgraph with more than one hybrid node.

As far as the converse implication goes, let $N$ be a 1-nested network where no hybrid node is the split node of any reticulation cycle. Let us assume that $N$ contains some biconnected subgraph, and in particular some minimal undirected cycle in the sense of Remark 2, with more than one hybrid node, and let us see that this leads to a contradiction. This will prove that $N$ is level-1.

The minimal undirected cycle of $N$ with at least two hybrid nodes cannot be a reticulation cycle, because no reticulation cycle in $N$ contains any hybrid node other than its end: the split node of a reticulation cycle in $N$ cannot be hybrid by assumption, and no intermediate node of a reticulation cycle in $N$ can be hybrid by Lemma 3. Therefore, this minimal undirected cycle will consist of $2k$ paths, with $k \geq 2$,

$$v_1 \leadsto h_1, v_1 \leadsto h_2, v_2 \leadsto h_2, v_2 \leadsto h_3, v_3 \leadsto h_3, v_3 \leadsto h_4, \ldots, v_k \leadsto h_k, v_k \leadsto h_1.$$  

Applying Lemma 1 to the paths $v_1 \leadsto h_1$ and $v_k \leadsto h_1$, we obtain that at least one of the nodes $v_1$ or $v_k$ is an intermediate node in a reticulation cycle for $h_1$. Assume that $v_1$ has this property (if $v_1$ was not intermediate in a reticulation cycle for $h_1$, then $v_k$ would be so, and we would traverse the cycle in the reverse sense). Then, applying Lemma 1 to the paths $v_1 \leadsto h_2$ and $v_2 \leadsto h_2$, and recalling that $v_1$ cannot be an intermediate node of a reticulation cycle for $h_2$ (because it is already so for $h_1$), we deduce that $v_2$ is an intermediate node of a reticulation cycle for $h_2$ and a descendant of $v_1$. Now, applying Lemma 1 to the paths $v_2 \leadsto h_3$ and $v_3 \leadsto h_3$, and since $v_2$ cannot be an intermediate node of a reticulation cycle for $h_3$, we deduce that $v_3$ is an intermediate node of a reticulation cycle for $h_3$ and a descendant of $v_2$, and hence of $v_1$. Repeating this process, when we reach $v_k$, we obtain that it must be an intermediate node of a reticulation cycle for $h_k$ and a descendant of $v_1$. But then, $v_k$ cannot be intermediate in the first reticulation cycle for $h_1$, and therefore $v_1$ must be a descendant of $v_k$, which yields a contradiction.

One possible way to forbid hybrid split nodes is to impose that the hybrid nodes have out-degree 1. This is usually done when hybrid nodes represent reticulation events (like hybridizations, recombinations, or horizontal gene transfers): the only
child of a hybrid node represents then the species resulting from the reticulation event.

**Corollary 1.** *Every 1-nested hybrid-1 network is level-1.*

In [1, Lem. 3] we proved that galled trees without out-degree 1 tree nodes are tree-child, that is, that every internal node in a galled tree has some child of tree type. A suitable modification of the argument used therein proves the following result.

**Proposition 2.** *Every 1-nested (and, hence, every level-1) network without out-degree 1 tree nodes is tree-child.*

**Proof.** Let \( N \) be a 1-nested network and let \( v \) be an internal node. There are two cases to consider.

On the one hand, if \( v \) has only one child, then this child is a tree node. Indeed, by assumption, if \( v \) has only one child, then \( v \) must be hybrid. But then, since 1-nested networks cannot contain hybrid nodes that are intermediate in reticulation cycles (Lemma 3), if the child \( w \) of \( v \) is hybrid, \( v \) must be the split node of a reticulation cycle for \( w \), and hence it must have at least two children.

On the other hand, if \( v \) has more than one child, then some child is a tree node. Indeed, assume that \( v \) has two hybrid children \( h_1 \) and \( h_2 \). Then, the 1-nested condition entails that \( v \) cannot be intermediate in reticulation cycles for both of them, and therefore it must be the split node of a reticulation cycle for at least one of them, say for \( h_1 \). But then some other child of \( v \) must be intermediate in this reticulation cycle, and this child must be of tree type, again by Lemma 3.

### 4 Results for 2-Hybrid Networks

Let us consider now the case when hybrid nodes have in-degree 2, in which case we can include galled and weakly galled trees in our discussion.

**Lemma 4.** *In a 2-hybrid 1-nested network, each hybrid node is the end of only one reticulation cycle.*

**Proof.** Let \( N \) be a 2-hybrid 1-nested network, and assume that it contains two reticulation cycles \( C, C' \) for a hybrid node \( h \), with split nodes \( w_1 \) and \( w_2 \), respectively. Let \((v_1, h), (v_2, h)\) be the pair of arcs with head \( h \). Then, in each reticulation cycle for \( h \), one merge path ends in \((v_1, h)\) and the other in \((v_2, h)\). Let \( P_{1,1} : \hspace{1em} w_1 \sim v_1 \rightarrow h \) and \( P_{2,1} : \hspace{1em} w_2 \sim v_1 \rightarrow h \) be the merge paths of \( C \) and \( C' \), respectively, ending in \((v_1, h)\), and let \( u \) be the first node in \( P_{1,1} \) and \( P_{2,1} \) such that the subpaths \( u \sim h \) of \( P_{1,1} \) and \( P_{2,1} \) are the same. If \( u \) is intermediate in both merge paths, this means that \( u \) has two different parents (one in each path) and therefore that it is hybrid, which contradicts Lemma 3. Therefore there are three possibilities: either \( u = w_1 = w_2 \), and then the paths \( P_{1,1} \) and \( P_{2,1} \) are the same, or \( u = w_1 \) and it
is intermediate in $P_{2,1}$, and then $P_{1,1}$ is a subpath of $P_{2,1}$, or $u = w_2$ and it is intermediate in $P_{1,1}$, and then $P_{2,1}$ is a subpath of $P_{1,1}$. In particular, $w_1$ and $w_2$ are either equal or connected by a piece of a merge path.

Using the same reasoning, we conclude that, if $P_{1,2} : w_1 \rightsquigarrow v_2 \rightarrow h$ and $P_{2,2} : w_2 \rightsquigarrow v_2 \rightarrow h$ are the merge paths of $C$ and $C'$, respectively, ending in $(v_2, h)$, then either $P_{1,2} = P_{2,2}$, or $P_{1,2}$ is a subpath of $P_{2,2}$, or $P_{2,2}$ is a subpath of $P_{1,2}$. Now all combinations yield to contradictions: if $w_1 = w_2$, then $P_{1,1} = P_{2,1}$ and $P_{1,2} = P_{2,2}$ and hence $C = C'$; if $w_1$ is a proper descendant of $w_2$, then $w_1$ is intermediate in $P_{2,1}$ and $P_{2,2}$, and then these paths are not internally disjoint; and if $w_2$ is a proper descendant of $w_1$, then $w_2$ is intermediate in $P_{1,1}$ and $P_{1,2}$, and these paths are not internally disjoint.

**Proposition 3.** A 2-hybrid network is 1-nested if, and only if, it is a weakly galled tree.

**Proof.** Let $N$ be a 2-hybrid 1-nested network, and assume that two reticulation cycles $C, C'$ share one arc $(u, v)$; by the previous lemma, these reticulation cycles have different ends, say $h$ and $h'$, respectively. Now, neither $u$ nor $v$ are intermediate in both cycles, because it would contradict the 1-nested condition. Therefore $u$ must be the split node of one of the cycles, say $C$, and $v$ must be $h$ or $h'$: but if $v = h$, then it is intermediate in $C'$, and if $v = h'$, then it is intermediate in $C$, and neither one thing nor the other is possible, by Lemma 3. This shows that $N$ is a weakly galled tree.

As far as the converse implication goes, let $N$ be a weakly galled tree and assume that two reticulation cycles $C, C'$ of $N$ share an intermediate node $v$. If $v$ were a hybrid node, then $C$ would share an arc with some reticulation cycle with end $v$ (both arcs ending in $v$ belong to any reticulation cycle for $v$, and one of them would belong to $C$), which would contradict the weakly galled tree condition. Then, $v$ must be a tree node. But in this case the only arc with head $v$ must belong to $C$ and $C'$, and hence these reticulation cycles share an arc, which is again impossible.

**Corollary 2.** Every galled tree is a level-1 network.

**Proof.** Every galled tree is a weakly galled tree, and hence 1-nested by Proposition 3, and it cannot have any hybrid split node, because different reticulation cycles cannot have any node in common. Then, Theorem 1 applies.

**Corollary 3.** In the semibinary case, level-1 networks, 1-nested networks and weakly galled trees are the same.

**Proof.** It is a direct consequence of Propositions 1 and 3, and Corollary 1.

**Remark 4.** Not every 2-hybrid 1-nested network is level-1: see network (b) in Fig. 1. And not every semibinary level-1 network is a galled tree: see network (a) in Fig. 1.
Proposition 4. In the binary case, level-1 networks, 1-nested networks, weakly galled trees, and galled trees are the same.

Proof. By Corollaries 2 and 3, it is enough to prove that every binary 1-nested network is a galled tree. So, let $N$ be a binary 1-nested network, and assume that two reticulation cycles $C, C'$ share one node. By Lemma 4 we know that $C$ and $C'$ have different hybrid ends, say $h$ and $h'$. In particular, they do not share their hybrid end. Moreover, the node they share cannot be intermediate in both cycles either, because $N$ is 1-nested. Let us see that all the other possibilities also lead to a contradiction:

- The hybrid end of one of the cycles cannot be the split node of the other, because split nodes cannot have out-degree 1 and hybrid nodes in binary networks have out-degree 1.
- The hybrid end of one of the cycles cannot be intermediate in the other, because of Lemma 3.
- If the split node of one of the cycles, say $C$, belongs to the other cycle, then (since it cannot be its hybrid end), one of its children in $C$ must be its child in $C'$, otherwise the split node would have out-degree 3. Now, this shared child of the split node of $C$ cannot be the hybrid end of $C$ or $C'$ (if it were the hybrid end of one of the cycles, it would be an intermediate hybrid node of the other cycle, against Lemma 3). Therefore, the shared child of the split node of $C$ will be intermediate in $C$ and in $C'$, which is prevented by the 1-nested condition.

5 Conclusion

In this paper we have established the actual relationships between the classes of galled trees, weakly galled trees, level-1 networks, and 1-nested networks. Our main results are summarized as follows:

(a) For arbitrary networks, \( \text{level-1} \iff \text{1-nested} \)

(b) For hybrid-1 networks, \( \text{level-1} \iff \text{1-nested} \)

(c) For 2-hybrid networks, \( \text{galled tree} \iff \text{level-1} \iff \text{1-nested} \iff \text{weakly galled tree} \)

(d) For semibinary networks, \( \text{galled tree} \iff \text{level-1} \iff \text{1-nested} \iff \text{weakly galled tree} \)

(e) For binary networks, \( \text{galled tree} \iff \text{level-1} \iff \text{1-nested} \iff \text{weakly galled tree} \)
So, if we restrict ourselves to 2-hybrid networks, we see that the node-disjoint reticulation cycles condition is the most restrictive one and that 1-nested networks are the most general, being equal to those networks with arc-disjoint reticulation cycles. So, since these networks have the same combinatorial properties as galled trees [11], from a formal point of view they are probably the right notion of “phylogenetic network with isolated reticulation cycles”. However, the distinction between node-disjoint and arc-disjoint reticulation cycles is very important in practice, because the assumption of no back or recurrent mutations entails that all nodes are labeled by different sequences and then, two arc-disjoint, but not node-disjoint, reticulation cycles cannot be torn apart by just duplicating any common nodes.

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